Study of α-amylase and lipase enzymes of awassi sheep fed by levels of dietary protein and probiotic additives

Amal F. Al-Abedi[1], Ali A. Saeed[1] and Khalida S. Al-Huosyney[2]

[1]Animal production department, college of agriculture, University of Al-Qasim green.

[2] Directorate of agriculture of Babylon.

Email of corresponding author: amalfaisal2228@gmail.com

Abstract:

Enzymes are naturally produced by living cells to cause specific biochemical reactions to catalyze the catabolic reactions by which substrates are digested into substrates' chemical compounds. These simple compounds are used in turn for cell growth. α-amylase and lipase are considered important enzymes for this process. The present study was carried out to investigate the effect of feeding Awassi lambs two levels of dietary protein and addition of probiotic (live Saccharomyces cerevisiae more than 3.0 × 1013 CFU/g and Bacillus subtilis more than 4.0 × 109 CFU/g) on α-amylase and lipase serum concentrations. Sixteen Awassi lambs were used in this study. Animals grouped into four treatments (four animals per each group). Lambs in the first treatment were fed concentrate diet containing 12% crude protein (CP) without probiotics. In the second treatment lambs were fed concentrate diet containing 12% CP plus 1 kg/ton of probiotic. In the third treatment lambs were fed concentrate diet containing 14% CP without probiotic. In the four treatment lambs were fed concentrate diet containing 14% CP plus 1 kg/ton of probiotic. Results showed that there is a significant decrease ($P<0.01$) in the α-amylase concentration due to increasing CP level in concentrate diet from 12 to 14%. Regarding the interaction treatments, higher ($P<0.05$) α-amylase concentration was detected in serum of lambs fed lower level of CP (12%) with or without probiotic as compared with those fed higher level of CP (14%) with or without probiotic. However no significant difference was observed between lambs fed 12 and 14% CP with probiotic. Regarding lipase, no significant differences were shown whether among the main effects or interaction treatments. We could conclude that, in the effect of main factors, α-amylase concentration was increased by lower dietary protein CP level (12%) was fed . In the interaction treatments, α-amylase concentration was increased when lower dietary protein CP (12%) with or without probiotic. Concerning lipase, serum concentrations were not significantly affected by level of dietary CP or interaction with addition of probiotic .

Keywords: Enzymes-Ruminants-Yeast-Bacteria-Awassi sheep

دراسة انزيمي الاميليز و اللايبيز للاغنام العواسي المغذاة بتراكيز مختلفة من البروتين الغذائي مع اضافة المعزز الحيوي

آمال فيصل العابدي ، علي امين سعيد، خالدة صالح الحسيني

[1] **قسم الثروة الحيوانية، كلية الزراعة، جامعة القاسم الخضراء .**

[2] **دائرة زراعة بابل.**

البريد الالكتروني:amalfaisal2228@gmail.com

المستخلص:

تنتج الانزيمات طبيعياً بوساطة الخلايا الحية لتؤدي دورها في التفاعلات البايوكيميائية. اذ انها تعمل على تحفيز تفاعلات الهدم لهضم المواد الى مركبات ابسط مثل السكريات والاحماض الامينية والاحماض الدهنية لتساعد على نمو خلايا الاحياء المجهرية التي تعيش في المضيف وخلايا المضيف نفسه، ويعد انزيم الأميليز و اللايبيز من الانزيمات المهمة في هذه العملية. اجريت الدراسة الحالية للتحري عن تأثير تغذية مستويين من البروتين الغذائي مع المعزز الحيوي (الخميرة الحية Saccharomyces cerevisiae اكثر من 3×10^{13} وبكتيريا bacillus subtilis اكثر من 4×10^{13} وحدة مكونة للمستعمرات.غم$^{-1}$) على تركيز انزيمي الأميليز و اللايبيز في مصل الدم في الحملان العواسية. اجريت الدراسة في قسم الانتاج الحيواني التابع الى كلية الزراعة/جامعة القاسم الخضراء للفترة من 2018/11/8 الى 2019/2/13 واستخدم فيها ستة عشر حيوان، وزعت الى اربعة معاملات (اربعة حملان في كل معاملة). غذيت الحملان في المعاملة الاولى على العلف المركز 12% بروتين خام بدون اضافة معزز حيوي، في المعاملة الثانية على العلف المركز 12% بروتين خام مع 1 كغم.طن$^{-1}$ من المعزز الحيوي، في المعاملة الثالثة على العلف المركز 14% بروتين خام بدون اضافة معزز حيوي، في المعاملة الرابعة على العلف المركز 14% بروتين خام مع 1 كغم طن$^{-1}$ من المعزز الحيوي. اظهرت النتائج وجود انخفاض معنوي ($P<0.01$) في تركيز انزيم الأميليز نتيجة لزيادة مستوى البروتين الخام. وبالنسبة الى تأثير التداخل سجل اعلى ($P<0.05$) تركيز للاميليز في مصل الحملان التي غذيت على مستوى البروتين الخام 12% مع او بدون اضافة المعزز الحيوي مقارنة مع مستوى 14% مع او بدون اضافة المعزز الحيوي. فيما يتعلق بأنزيم اللايبيز لم يلاحظ وجود اختلافات معنوية سواء بتأثير العاملين الرئيسيين او التداخل بينهما. ويمكن الاستنتاج بان تركيز الأميليز قد انخفض عند تغذية المستوى المنخفض من البروتين الخام، وبتأثير التداخل سجل تركيز الأميليز ارتفاعا عند تغذية المستوى المنخفض من البروتين الخام مع او بدون اضافة المعزز الحيوي. ولم يتأثر تركيز اللايبيز بتأثير العاملين الرئيسيين او التداخل بينهما

الكلمات المفتاحية: انزيمات–مجترات– خميرة– بكتيري–الأغنام العواسي.

Introduction:

Probiotic is a collection of useful microbes mixed with feeds to make a helpful and healthy microbial balance in the intestine (Nunes, 1994). It also has been defined as live microorganisms that when administrated at proper doses will give a flora (Rask,

Adlerberth, Berggren, & Ahrén, 2013). However, probiotic seemed to improve animal performance (Hassan , 2009), weight gain (Hassan & Tawffek, 2009), and feed conversion ratio (El-Shaer, 2003) egg quality (Jasim, 2017). It affects the animal by improving its intestinal balance and produces a condition that holds the harmful microorganisms back and supports helpful ones (Line, Bailey, Cox, & Stern, 1998). It works to keep the health by reducing diseases through lowering a pathogens proliferation, enhancing the immunity and resistance against the infections (Mountzouris & Tsirtisikos, 2007).

Probiotics are essential materials that can increase the growth rate and productive performance (AI-Jassim, AL-Ani, Hassan, & Dana, 1991). (Fuller, 1989) has shown that using Lactobacillus probiotics in animal feed brings about improving production of enzymes that supports digestion and increases in growth, but Al-Samarrai, Ahmad, Al-Mashhadani, & Abbas (2014) studied the effect of addition of Iraqi probiotic on blood parameters in Awassi lambs feeding barley straw, they noticed that amylase concentration was decreased when probiotic used at high level of probiotic. They also observed that probiotic decreases serum cholesterol concentration. Santose & Tanaka (1995) noticed that *Bacillus subtilis* probiotic decreases the activity of the acetyl-coA carboxylase which is the restricting enzyme in fatty acid synthesis.

Nutritional regulation of digestive enzymes has received limited study in ruminants. Studies on the exact regulatory mechanisms in ruminants are lacking (David, 1993). Ruminants have the same digestive enzymes as do non-ruminants to perform digesting and absorbing processes within the intestine (Church, 1979).

Carbohydrates digestion takes place in the duodenum. Recall that chyme from the stomach enters the duodenum and mixes with digestive secretion from pancreas, liver, and gallbladder. Pancreas juices contain amylase which continue the breakdown of starch and glycogen into maltose (a disaccharide). The disaccharides are broken down into monosaccharides by enzymes called maltase, which breaks down maltose into glucose (Hamasalim, 2015).

Ruminants don’t have salivary α-amylase (Church, 1979) but secrete pancreatic α-amylase which hydrolyzes starch within the intestinal lumen (Kreikemeier K. K., 1991). The data obtained from Russell & Young, (1981) demonstrated that a pancreatic α-amylase concentration increases with high intake of grain diet.

The bulk of lipid digestion occurs in the small intestine due to pancreatic lipase. When chyme enters the duodenum, the hormonal responses trigger the release of bile acids in the digestion of lipids by emulsification. Emulsification is a process in which large lipid globules are broken down into several small lipid globules. Pancreatic juices contain enzymes called lipase, if the lipid in the chyme aggregates into large globule, the very little surface area of the lipids is available for the lipases to act on, leaving lipid digestion incomplete (Hamasalim, 2015).

In ruminants, Pancreatic lipase has the same nature and function of nonruminants (Noble, 1978). The changes in lipase are mediated through changes in circulating B-hydroxybutyrate (David, 1993). Brannon, (1990) reported that in nonruminants pancreatic lipase increases in response to increased dietary triglyceride. Johnson (1973)

noticed secretion of pancreatic juice and lipase in sheep that are infused with safflower or coconut oils decreased both pancreatic juice and lipase secretion. Mustafa (2019) showed that eucalyptus oil increases lipase. All the previous studies have overlooked the study of the impact of different levels of dietary protein and probiotic additives on amylase and lipase concentrations. As a result, the present study addresses the effect of dietary protein and probiotic additives on amylase and lipase concentrations.

Materials and methods

Experimental diets:

Experimental feed comprised of concentrate and roughage diets. Concentrate diets were prepared by mixing their ground ingredients (wheat barn, barley, yellow corn, and soybean meal) after performing their chemical analysis. These diets were prepared locally with the addition of salt, vitamins and mineral mix and probiotic at specific levels of 0 and 1 kg/ton of diet as recommended by the manufacturer. Two concentrate diets were prepared on the basis of crude protein (CP) levels of 12 and 14% on dry matter (DM) basis. In each concentrate diets, levels of ingredients were estimated to secure the standard ratio of rumen degradable nitrogen (RDN) to metabolizable energy (ME) of about 1.34g RDN/MJ of ME (ARC, 1984). Roughage was ground wheat straw. Concentrates were offered to lambs at 2.5% of body weight, whereas, straw was offered on *adilbitum* basis. Quantities of concentrate diet offered to each lamb were adjusted weekly according to changes in their body weights. Table 1 shows levels of ingredients in concentrate diets. Table 2 shows the chemical compositions of these ingredients, concentrate diets, and wheat straw.

Table 1: Components of concentrate diet (%)

Concentrate diets	barley	WB	YC	SBM	Urea	NaCl	V-M
12%CP	58	30.75	8	0.63	0.62	1	1
14% CP	37	35	23	2.26	0.74	1	1

WB, Wheat bran; YC, Yellow corn; SBM, Soybean meal; V-M, Vitamin-minerals mix

Table 2: Chemical composition of concentrate ingredients, concentrate diets and wheat straw

Diets and ingredients	DM (%)	% of DM					
		Ash	OM	CP	EE	CF	NFE
Barley	91.78	5.65	94.35	10.16	1.99	6.71	75.49
Wheat bran	91.75	5.48	94.52	14.27	3.77	13.96	62.52
Yellow corn	91.18	2.22	97.78	9.27	3.51	4.2	80.80
Soybean meal	91.93	7.87	92.03	45.48	1.83	5.37	39.35
Urea	-	-	-	287.5*	-	-	-
Concentrate 1	88.11	5.69	94.31	12.53	3.39	4	74.39
Concentrate 2	88.44	5.79	94.21	14.51	3.13	4.14	72.43
Wheat straw	88.07	7.09	92.91	3.22	1.86	37.69	51.14

DM, Dry matter; OM, Organic matter; CP, Crude protein; EE, Ether extract; CF, Crude fiber; NFE, Nitrogen free extract; * 46× 6.25

Experimental animals:

Sixteen Awassi sheep were used in this study. Animals were bought from the local market with average body weight of 18.76±2.35kg and 4-6 months of age. Lambs were transferred to the place of the study and viewed to veterinary tests to ensure its safety and presence of disease. Lambs were injected with Vilmectin against external parasites (3ml/sheep). Lambs were also drenched with Vinazole against intestine and liver worms (35ml/100kg BW). Gentarilin Fort was used (5ml/sheep) to treat diarrhea single case. Sodium carbonate was used in limited cases to overcome nutritional problems associated with high consumption of concentrate in few cases. Lambs were weighed using digital balance and randomly allotted into 4 treatments with 4 lambs per each. Lambs were housed in individual pens provided with separated containers for concentrate diets, wheat straw and clean water. Lambs were gradually adapted to consume concentrate diets before the start of the study. The preliminary period was ranged 10-20 days. Concentrate diets were offered twice a day, morning meal at 8 AM and evening meal at 4 PM**.**

Sampling and measurements:

Samples of blood were collected during the tenth week of the experiment from each animal. Blood was withdrawn from the jugular vein by a needle with a syringe (5ml), transferred into plain tubes and centrifuged (5000r/m. for 5 minutes) to separate the serum. The serum was collected by micropipette and transferred into Eppendorf tubes and stored at -20◦C until enzymatic investigation was performed. After 3 days of serum collection, the enzymatic investigations of α-amylase and lipase have been measured using Bio-base apparatus and Spinreact kits for enzymes [Sprinreact. S.A.U. citrasanta Coloma.717176 santesterede bas-(Girona) Spain].

Statistical analysis:

The data was statistically analyzed according to factorial experiments (2 × 2) in completely randomized design (CRD) to evaluate the effect of the main factors studied in the experiment and interaction between them. Statistics analysis was performed with SAS (2010).

Results and Discussion:

Table 3 shows the main effect of dietary protein level of CP and addition of probiotic on serum concentration of α-amylase. Results revealed that there was a significant decrease ($P<0.01$) in α-amylase serum concentration from 27.92 to 18.45 units due to increasing dietary levels of CP from 12 to 14%, but it was not significantly affected by addition of probiotic. Regarding effect of interaction between those two main factors, table 4 shows that higher ($P<0.05$) α-amylase serum concentration was detected when lambs were fed lower level of CP without and with addition of probiotic (29.23 and 26.62 units) as compared with those fed higher level of CP without and with addition of probiotic (13.47 and 18.85 units).

Table 3: Effect of dietary protein levels and addition of probiotic on serum concentration of α-amylase (Units ± SE)

Enzyme	Level of dietary protein (%)		Addition of probiotic, kg/tone	
	12	14	0	1
α-amylase	27.92 ± 2.21[a]	18.45 ± 3.02[b]	21.35 ± 3.20	22.73 ± 3.03

a, b= significant differences at probability level (P<0.01)

Table 4: Effect of interaction between dietary protein levels and addition of probiotic on serum concentration of α-amylase (Units ± SE)

Level of dietary protein (%)	12		14	
Addition of probiotic, kg/tone	0	1	0	1
α-amylase	29.23[a] ± 2.32	26.62[ab] ± 3.02	13.47[c] ± 1.05	18.85[bc] ± 5.00

a, b= significant differences at probability level (P<0.05)

We know that increases in α-amylase concentration with increased intake correspond with the controlling mechanism for nonruminants whereby glucose and insulin interaction to control pancreatic α-amylase production (Kreikemeier, Harmon, Peters, Gross, & Amendariz, 1990). Similarly, David (1993) has shown that an arterial concentration and a hepatic extraction of insulin increased as intakes increased of both 75% alfalfa and 75% corn and SBM diets were increased .

There is inconsistence between the data obtained in a present study with that described in the literature in the sense that an α-amylase concentration in the pancreatic secretion increases with carbohydrate intake, glucose infusion increases α-amylase secretion, maltose infusion didn't change a pancreatic α-amylase secretion and starch infusion decreased a pancreatic α-amylase secretion (Snook, 1971 and Coning, 1977).

This result was not the first indication that increased small intestinal carbohydrate may affect pancreatic α-amylase either positively or negatively. Moreover, there are other factors that affect pancreatic secretion like minerals as asserted by Brannon & Collins (1987) where pancreatic α-amylase concentration was enhanced with manganese deficiency and this result initiated by increasing pancreatic α-amylase mRNA (Chang & Brannon, 1990). On the other hand, Lewis, Fields, Craft, & Yang (1987) found that low dietary Cu decreases α-amylase, lipase and trypsin.

Regarding lipase, results haven't shown any significant differences between main effects or interaction between them as shown in table 5 and 6 show.

Table 5: Effect of dietary protein levels and addition of probiotic on serum concentration of lipase (Units ± SE)

Enzyme	Level of dietary protein (%)		Addition of probiotic, kg/tone	
	12	14	0	1
lipase	21.22 ± 1.34	23.30 ± 2.20	23.82 ± 2.13	20.70 ± 1.31

Table 6: Effect of interaction between dietary protein levels and addition of probiotic on serum concentration of lipase (Units ± SE)

Level of dietary protein (%)	12		14	
Addition of probiotic, kg/tone	0	1	0	1
lipase	22.92 ± 1.70	19.52 ± 1.89	24.72 ± 4.22	21.88 ± 1.86

As in α -amylase, there are contrastive studies about lipase. Brannon, 1990) reported that in nonruminants pancreatic lipase increases in response to increased dietary triglyceride. It was noticed that secretion of pancreatic juice and lipase in sheep that are infused with safflower and coconut oils lowered both pancreatic and lipase secretion (Johnson, 1973).

References

AI-Jassim, R., AL-Ani, A. N., Hassan, S., & Dana, T. K.-J. (1991). Effects of dietary supplementation with rumen undegradable protein on carcass characteristics of Iraqi Awassi lambs and desert goats. *4*, 269-275.

Al-Samarrai, W., Ahmad, A. R., Al-Mashhadani, N. I., & Abbas, S. M. (2014). Effect of addition of Iraqi probiotic on blood parameters in awassi lambs feeding barley straw. *Global Journal of Biochemistry and Biotechnology, 3*, 84-90.

Al-Samarrai, W., Ahmad, A. R., Al-Mashhadani, N. I., & Abbas, S. M. (2014). Effect of addition of Iraqi probiotic on blood parameters in Awassi lambs feeding barley straw. *Global Journal of Biochemistry and Biotechnology, 3*, 84-90.

ARC (1984). The nutrient requirements of ruminant Livestock. Commonwealth Agricultural Bureau, Slough. UK**.**

Brannon, P. M. (1990). Adaption of the exocrine pancreas to diet. *Annual Review of Nutrition, 10*, 85-105.

Brannon, P., & Collins, V. P. (1987). Alterations of pancreatic digestive enzyme contents in the manganese-deficient rat. *Journal of Nutrition, 117*, 305-311.

Chang, S. C., & Brannon, P. M. (1990). Effect of dietary manganese deficiency on rat pancreatic α-amylase mRNA levels. *Journal of Nutrition, 120*, 1228-1234.

Church, D. G. (1979). *Digestive Physiology and Nutrition of Ruminants* (Vol. 2nd). USA, Oregon: O&B Books press.

Coning, T. (1977). The possible role of hydrolysis products of the dietary components in the mechanisms of the exocrine pancreatic adaptation to the diet. *World Review of Nutrition and Dietetics, 27*, 132-144.

David, L. H. (1993). Nutritional regulation of post ruminal digestive enzymes in ruminants. *Journal of Dairy Science, 76*, 2102-2111 .

El-Shaer, E. H. (2003). Nutritional Studies in Ruminants (Effect of Yeast Culture Supplementation and Concentrate: Roughage Ratio on Performance of Growing Lambs. Faculty of Agric., Univ. of Mansoura. Mansoura: Univ. of Mansoura.

Fuller, R. (1989). Probiotics in man and animals. *Journal of Applied Bacter*iology, 66, 365-378.

Hamasalim, H. J. (2015). The impact of some widely probiotic (Iraqi probiotic) on health and performance. *Journal of Biosciences and Medicines, 3*, 25-36.

Hassan, S. a. (2009). The effect of supplementation of medicinal plants and probiotic on growth rate and some blood parameters of Karadi lambs. *Egyptian Journal of Nutrition and Feeds, 12*, 53-63.

Hassan, S., & Tawffek, J. A.S. (2009). Effect substitution gradually percentages of reed silage with alfalfa hay fed with probiotic to Awassi lamb on carcass characteristics. *The Iraqi Journal of Agricultural Sciences, 40*, 138-147.

Jasim, M. S. (2017). Effect of supplementation of probiotic and citric acid to laying hens diet on eggs quality traits. *Iraqi Journal of Agricultural Sciences, 48*, 985-952.

Johnson, T. (1973). *Ovine and Avian Pancreatic Lipase Response to Source and Level of Dietary Fat.* Kentucky: Univ. of Kentucky.

Kreikemeier, K. K. (1991). Effect of abomasal carbohydrate infusion on ileal digesta oligosaccharide flow in steers. *Journal of Animal Science, 69*, 517.

Kreikemeier, K. K., Harmon, D. L., Peters, J. P., Gross, K. L., & Amendariz, C. K. (1990). Influence of dietary forage and feed intake on carbohydrase activities and small intestinal morphology of calves. *Journal of Animal Science, 68*, 2916-2929.

Lewis, C., Fields, M., Craft, N., & Yang, C. Y. (1987). Alteration of pancreatic enzyme activities in the small intestine of rats fed high fructose, low copper diet. *Journal of Nutrition, 117*, 1447-52.

Line, E., Bailey, S. J., Cox, N. A., & Stern, N. J. (1998). Effect of yeast-supplemented feed on salmonella and campylobacter populations in broilers. *Journal of Poultry Science, 77*, 405-410.

Mountzouris, K., & Tsirtisikos, P. a. (2007). Evaluation of the efficacy of a probiotic containing Lactobacillus, Bifidobacterium, Enterococcus and Pediococcusstrains in promotion broiler performance and modulation cecal microflora composition and metabolic activities. *Journal of Poultry Science, 86*, 309-317.

Mustafa, M. A. (2019). Effect of eucalyptus leaves and its supplementation with diet on broiler performance, microbial and physiological statues to alleviate cold stress. *Iraqi Journal of Agricultural Sciences, 50*, 953- 963.

Noble, R. (1978). Digestion, absorption and transport of lipids in ruminant animals. *Progress in Lipid Research, 17*, 55-91.

Nunes, C. (1994). Microbial probiotics and their utilization in husbandry. *Revista Portuguesade Ciencias Veterinarias, 89*, 166-174.

Rask, C., Adlerberth, I., Berggren, A., & Ahrén, I. L. (2013). Differential effect on cell-mediated immunity in human volunteers after intake of different lactobacilli. *Journal of Clinical & Experimental Immunology, 172*, 321-332.

Russell, J., & Young, A. W. (1981). Effect of dietary corn starch intake on pancreatic amylase and intestinal maltase and pH in cattle. *Journal of Animal Science, 52*, 1177-1182.

Santose, U., & Tanaka, K. a. (1995). Effect of dried *Bacillus subtilis* culture on growth, body composition and hepatic lipogenic enzyme activity in female broiler chicks. *British Journal of Nutrition, 74*, 523-529.

Snook, J. T. (1971). Dietary regulation of pancreatic enzymes in the rat with emphasis on carbohydrate. *American Journal of Physiology, 221*, 1383-7.