

Measuring of serum pepsinogens level in abomasal lesions of sheep

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Abstract

The study aimed to investigate serum pepsinogens values with and without abomasal lesions of sheep. Eighty five blood and abomasal samples containing abomasal lesions were collected during slaughtering of sheep in slaughterhouse of Al-Qasim city-Iraq. The abomasal mucosa was examined, and the type, number, and location of lesions were recorded. Serum was taken for pepsinogen assay by ELISA technique. Results revealed that the highest percentage of lesions in abomasum were nodules (48.23%), ulcers (23.52%), parasites (17.64%) and hemorrhage (10.58%). A significant difference ($P \leq 0.05$) was recorded between nodules and other abomasum lesions. Serum pepsinogens values in abomasal lesions was found higher (3.8) than those without abomasal lesions (3.13), and showed a significant difference in samples which had different lesions (3.8 ± 0.13) than free lesions samples (3.13 ± 0.1). No significant difference was showed between all samples containing ulcer, nodules and parasites (3.65 ± 0.28) (3.88 ± 0.24) (3.65 ± 0.95) respectively although the nodules was recorded higher serum pepsinogens comparative with other lesions.

Key words: Pepsinogens, sheep, ELISA, abomasum lesions, lesions.

قياس مستوى بيبسينوجين مصل الدم مع آفات المنفحة في الاغنام

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الخلاصة

هدفت الدراسة الى تقييم مستوى البيبسينوجين في مصل الدم بوجود آفات المنفحة في الاغنام وعدم وجودها. خمسة وثمانون عينة دم وعينة منفحة تحتوي على آفات جمعت اثناء ذبح الاغنام في مجزرة مدينة القاسم. فحصت مخاطية المنفحة وسجل نوع وعدد وموقع الآفات ، كما اخذ مصل الدم للتحري عن مستوى البيبسينوجين باستخدام تقنية الاليزا. أظهرت النتائج ان آفات العقد هي من اكثر الآفات تواجدا بالعينات المفحوصة (48.23%)، حيث سجلت فارق معنوي تحت مستوى احتمالية ($P \leq 0.05$) مع الآفات الاخرى والتي شملت وجود القرع (23.52%) ثم الطفيليات (17.64%) وقد سجلت النزوفات اقل نسبة وجود (10.58%). لوحظ ارتفاع مستوى البيبسينوجين في العينات التي تحتوي على آفات بمعدل (3.8) عن العينات الخالية من الآفات وبمعدل (3.13) ، حيث لوحظ وجود فارق معنوي سجل للعينات التي تحتوي آفات مختلفة مقارنة مع العينات التي لا تحتوي أي آفة مرضية. ولم يسجل أي فارق بين الاصابات المشتركة في العينات المفحوصة. **الكلمات المفتاحية:** بيبسينوجين ، الاغنام ، الاليزا ، آفات المنفحة ، الآفات.

Introduction

Abomasum affected primarily or secondarily by infections, parasites and foreign bodies, the common conditions are erosion and ulceration, these lesions such as gastric ulcers occurs in all ages of animals which occasionally cause acute hemorrhage of abomasum, along with indigestion and melena, and sometimes perforation of abomasum takes place, producing painful acute local peritonitis or acute diffuse

peritonitis with sudden death (1). In Iraq abomasal lesions are studied by Jassim and Alkhaled (2) who found that proportions of abomasal infection in sheep are five types of lesions; ulcers (21.4%), parasites (21.4%), hemorrhage (10.1%), thickness (8.9%) and nodules (12.5%). Pepsinogen is an inactive form of pepsin, which is the most important proteolytic enzyme of gastric juice. Increased activation of pepsinogen into pepsin by

enhanced acidity of gastric contents can cause ulcers in humans and animals (3). Scott *et al.* (4); McKellar *et al.* (5) were mentioned that pepsinogen is converted to pepsin at acidities below pH 5, the region of protein pH stability or instability shifts 2 pH units toward the acid side, as the increased pepsinogen content of the hyperplastic mucous neck cells appeared to compensate for the reduced content in mature chief cells. Failure of pepsinogen conversion to pepsin when the abomasal pH rises and to increased secretion of pepsinogen. Simpson *et al.* (6) reported that sheep which have very low serum pepsinogen levels when parasitized are likely to be those with very low tissue pepsinogen. Schaw *et al.* (7) found that increase in pepsinogen reflects mucosal damage as a consequence of an Ostertagia infection in cattle, whilst Zadnik and Mesaric (8) pointed out that elevation of pepsinogen levels induced by non-parasitic diseases in cows such as acute catarrhal abomasitis, abomasal ulcerations and left or right abomasal displacement are not confirmed. In Iraq and due to lack information about the effect of abomasal lesions on serum pepsinogens, so that the study was conducted to determination these effect.

Materials and methods

Collection of samples:

Eighty five blood and abomasal samples containing abomasal lesions were collected during slaughtering of sheep in slaughter-house of Al-Qasim city-Iraq.

Blood samples:

Blood samples were collected during slaughtering of animals from jugular vein in sterile jell tubes and placed diagonally and allowed to clot, then transported in ice box to the laboratory, and centrifuged to separated serum. Serum was put in eppendorf tubes and storage in deep freeze -20°C until use.

Abomasal samples:

Abomasal samples were collected immediately after slaughtering of animals, when separation of abomasum from its anterior part associated with omasum and its posterior part with small intestine after tying of both ends. Each sample was placed in nylon bags and transported immediately to

the laboratory in ice box. The abomasal mucosa was examined, and the type, number, and location of lesions were recorded.

Pepsinogen Assay:

This test was done according to (9). The procedure as follows:

Conical Eppendorf tubes size 1.5 ml used by added duplicated 50µl of serum was added to 250 µl of substrate solution (BSA 2%) in assay buffer (glycine-NaCl-HCl buffer) was prepared previously. The tubes was closed, and briefly vortex, and incubated at 37°C for 24 hours. After incubation the peptic digestion was arrested and the undigested substrate precipitated by the addition of 500 µl of trichloroacetic acid (4 g /L). Following vortexing and standing for 10 minutes, the tube was centrifuged for five minutes at 10,000 g in a bench top microcentrifuge. Three separate 20 aliquots of the supernatant were then transferred to wells of a flat bottomed microtitre plate and 200 µl of a 0.25 N NaOH solution was added to each well. After mixing for two minutes, 30 µl of diluted water (1:3 v/v). Folin and Ciocalteu's color reagent were added to each well. The microtitre plate was agitated for two minutes and incubated at room temperature (range 20-25°C) for 30 minutes. The optical density was then measured at 680 nm with an ELISA-reader. To calculate the amount of tyrosine produced, a set of tyrosine standard solutions of 0.1 umol ml⁻¹, 0.2 umol ml⁻¹ and 0.3 pmol ml⁻¹ was freshly prepared from a sterile stock solution (0.01 M L-tyrosine in 0.1 N HCl) and measure on the microtitre plate together with the supernatant estimating the pepsinogen concentration in sera of S1 with both techniques.

Statistical analysis:

Chi-square test was applied for the statistical analysis of the data, at (P≤0.05) level of significance (10).

Calculation of pepsinogen concentration with the micro method

$$U \text{ tyr} = (\text{OD sample} - 0.020) \times F \times 11.11$$

U tyr; denotes units of tyrosine: micro moles of tyrosine released per liter of serum per minute, OD; denotes the arithmetic mean optical density of the three wells; 0.020 is the

correction factor for the presence of tyrosine in un-incubated samples.

F; is the calculation factor derived from the standard curve prepared from the tyrosine standard.

$$F = \frac{0.1}{OD \text{ try } 0.1 \text{ mmol ml} - 1} + \frac{0.2}{OD \text{ try } 0.2 \text{ mmol ml} - 1} + \frac{0.3}{OD \text{ try } 0.3 \text{ mmol ml} - 1} / 3$$

Results

Three types of lesions were detected, as well as presence of parasites. The highest rates of abomasum lesions were nodules (48.23%), ulcers (23.52%), parasites (17.64%) and lowest lesion was hemorrhage (10.58%) (Fig. 1).

The serum pepsinogen values (IU/L) of animals have abomasal lesions found higher (3.8) than those without abomasal lesions (3.13). Higher significant means of pepsinogens values was recorded in samples had different lesions (3.8±0.13) than samples free from lesions (3.13±0.1). The highest mean values of serum pepsinogens was recorded in samples which have nodules

(3.88) but ulcer and parasites were recorded same means values (3.65). No significant difference was showed between all samples, the ulcer, nodules and parasites (3.65± 0.28) (3.88±0.24) (3.65±0.95) respectively. Also recorded significant difference between samples contains nodules and samples without lesions, this significant not recorded with samples contain ulcer and parasites. Serum pepsinogens of mixed abomasal lesions (ulcer, nodules, parasites and hemorrhage) were recorded (4.8) highest values, and lowest serum pepsinogen (3.36) was recorded in abomasal samples have two lesions (ulcer and nodules) (Fig. 2) (Table 1).

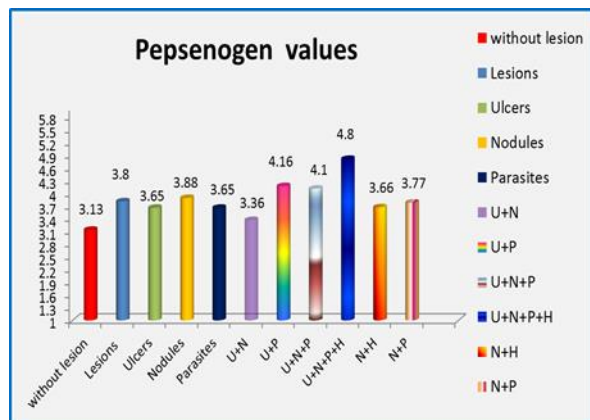
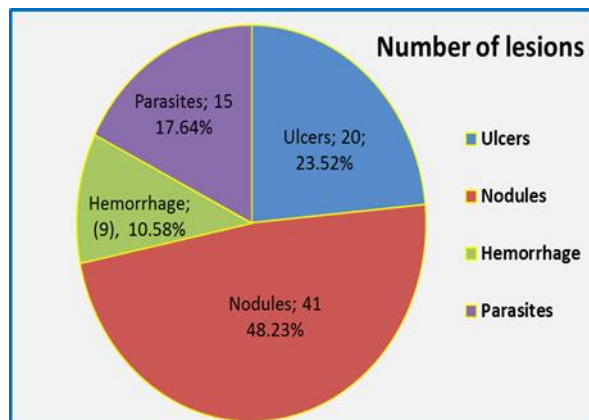


Fig. (1): Shows the numbers and proportion of abomasal lesions.

Fig. (2): Explain the serum pepsinogen values (IU/L) with and without abomasal lesions; (U: Ulcers, N: Nodules, P: Parasites, H: Hemorrhage)

Table (1): Explain the serum pepsinogen values (M±SE IU/L) of sheep.

No.	Without lesion	With lesions	ulcer	nodule	parasite	U+N	U+P	U+N+P	U+P+N+H	N+H	N+P
Pepsinogen Mean± SE	3.13 ± 0.19	3.8 ± 0.13	3.65 ± 0.28	3.88 ± 0.24	3.65 ± 0.95	3.36 ± 0.46	4.16 ± 0.35	4.1 ± 0	4.8 ± 0	3.66 ± 0.37	3.77 ± 0.52
	a	bc	ab	bc	ab	ab	c	bc	d	ab	bc

Similar letters refers to non-significant differences. Different letters refers to significant differences (p<0.05). U:Ulcers, N:nodules, P:parasites, H:Hemorrhage.

Discussion

The lesions were recorded in abomasum of sheep are nodules (48.23%), ulcers (23.52%), parasites (17.64%) and hemorrhage (10.58%). This results is differ than study conducted by (11) on prevalence of abomasal abnormalities in sheep in Iran, whose found the ulcers 75% and nodular lesions are 79%, while (2) found five abomasal infections in sheep, the lesions are predominantly ulcers and parasites with few being nodules, hemorrhage and few thickness. Ulcers (21.4%), parasites, (21.4%), mild to severe hyperemia (8.9%), thickness of abomasal wall was (10.1%) nodules (12.5%) these results compatible with result of our study excepted percentage of abomasal nodules have lowest than in current results. Other study conducted by (12) in abomasal lesions of cattle found many lesions in abomasum; ulcer and erosions (10.97%), hyperemia (9.7%), parasites (5.28%), lower than found in the current study. The use of serum pepsinogen activity promised to provide a simple serum test to diagnose abomasal ulcer. Argenzio (13); Morgado *et al.*(14) mentioned that pepsinogen is the inactive form of pepsin, and it is converted to pepsin in an acid environment. The conversion begins at a gastric pH of approximately 5.0, and its optimal activity occurs at pH values between 1.8 and 3.5. A high serum pepsinogen level is a good indicator of abomasal mucosa lesions, and animals with abomasal ulcers have higher pepsinogen concentrations than healthy animals. The normal serum values range from 0 to 5.0 IU/L. Kataria *et al.*(15) found that blood levels of pepsinogen can be used in the diagnosis of abomasal parasitism or disorders. Increase plasma levels of pepsinogen are due to its leakage into the blood vessels from damaged abomasal mucosa and increased activation of pepsinogen into pepsin by enhanced acidity of gastric contents can cause ulcers in

humans and animals. Also (16) mentioned that increased serum gastrin and pepsinogen concentrations and generalized histological changes are associated with parasites in the abomasal lumen. Irvine *et al.*(17) mentioned that nematode larvae developing within the glands cause local loss of parietal cells and mucous cell hyperplasia whereas reduced acid secretion, increased serum gastrin and pepsinogen concentrations and generalized histological changes are associated with parasites in the abomasal lumen. Berghen *et al.*(18) pointed out an increase in serum pepsinogen concentration reflects mucosal damage as a consequence of *O. ostertagi* infection. There is hypoplasia and metaplasia of the parietal cells resulting in a decrease in acid production and a subsequent reduction of the pepsinogen transformation into pepsin. The accumulated pepsinogen may escape into the blood between the broken cell junctional complexes. Nalini *et al.* (19) diagnosed pepsinogen in healthy sheep were 103.45 ± 10.41 pg/ml and 153.61 ± 13.21 mU tyrosine, respectively. In Hemonchus infected and drought affected sheep a significant ($p \leq 0.05$) increase was observed in the mean values for both the parameters in comparison to that of healthy, it highest values for both the parameters were observed in hemonchus infected animals and showed that feeding did not affect the levels of gastrin and pepsinogen. Mesaric (20) was found that significant influence of the extent and number of changes to the mucous membrane of the abomasum on the raised serum pepsinogen confirmed the statement that the concentration of serum pepsinogen is a good reflection of the damage to the abomasal mucosa. Paranagama *et al.*(21) evaluated the serum pepsinogen in goat infested with abomasal hemonchosis, who showed increase in serum pepsinogen concentration parallel to that of the hemonchus worm burden in the abomasum.

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