

USE OF PLASMA GASTRIN AND PEPSINOGEN LEVELS AS DIAGNOSTIC MARKERS OF ABOMASAL DYSFUNCTION IN MARWARI SHEEP OF ARID TRACT

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Summary: Hormone gastrin is secreted from gastrin cells of pyloric region of abomasum into the blood circulation, thence to reach the parietal cells and is an important stimulator of acid and pepsinogen secretion. Pepsinogen, a proenzyme is an inactive form of pepsin which is the most important proteolytic enzyme of gastric juice. To assess the role of gastrin and pepsinogen in the diagnosis of abomasal parasitism or disorders, the gastrin and pepsinogen levels were determined in the plasma of Marwari breed of sheep belonging to farmers' stock of arid tract of Rajasthan state, India. The animals, from which the blood samples collected, were grouped into healthy, haemonchus infected and drought affected. In healthy animals sampling was carried as one time random sampling and three times sampling. The overall mean values of plasma gastrin and pepsinogen in healthy Marwari sheep were 103.45 ± 10.41 pg/ml and 153.61 ± 13.21 mU tyrosine, respectively. In haemonchus 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 stock. The highest values for both the parameters were observed in haemonchus infected animals. The sampling time did not affect the gastrin and pepsinogen levels. This showed that feeding did not affect the levels of gastrin and pepsinogen. The responses of gastrin and pepsinogen in affected sheep indicated that they can be used as diagnostic markers in animals affected with abomasal dysfunctions. Probably the presence of parasites or inanimate objects in pica damaged the mucosa causing an impairment of abomasal function. Damage to mucosa is related with higher gastrin and pepsinogen release. Further the results suggested that one time random sampling can be carried out in suspected clinical cases for the determination of plasma gastrin and pepsinogen. The data obtained can be used as a base line for the future studies in this direction in Marwari sheep or other breeds of sheep.

Key words: drought; gastrin; *haemonchosis*; Marwari sheep; pepsinogen; pica

Introduction

Hormone gastrin is secreted from gastrin cells of pyloric region of abomasum into the blood circulation, thence to reach the parietal cells and is an important stimulator of acid and pepsinogen secretion (1). Pepsinogen, a proenzyme is an inactive form of pepsin which is the most important proteolytic enzyme of gastric juice. In the gastric or abomasal lumen pepsinogen is converted into pepsin in the

presence of acid (1, 2). In ruminant blood a certain physiological level of pepsinogen exists (3). Blood levels of pepsinogen can be used in the diagnosis of abomasal parasitism or disorders (4). The increased plasma levels of pepsinogen are caused due to its leakage into blood vessels from damaged abomasal mucosa (5). Increased activation of pepsinogen into pepsin by enhanced acidity of gastric contents can cause ulcers in humans and animals (6).

There are reports, although scant, on the use of plasma pepsinogen and gastrin as indicators of gastric dysfunctions in man (7, 8) and animals (4, 9, 10). However, there is no literature available on this as-

pect in Marwari breed of sheep. Also, there is rarity of literature regarding normal values of pepsinogen and gastrin in animals.

The Marwari sheep is an important breed of sheep in arid regions and frequently face problems of droughts (11). During drought periods animals feed on non-conventional feeds and develop pica in which condition they start eating in-animate objects (2). The gastrointestinal worm infestation is also a great problem in sheep. In these circumstances mucosa of abomasum is damaged greatly impairing digestive functions as proper gastric mucosa plays an important role in digestive processes. However, many a times damage to abomasal mucosa remains undiagnosed. The determination of plasma levels of gastrin and pepsinogen can be an important aid for taking timely measures to treat such animals (2, 5, 6). The present investigation was planned to explore the possibilities of role of plasma gastrin and pepsinogen as diagnostic tools in assessing abomasal involvement in Marwari sheep and to set the normal values of these parameters. In the field conditions to overcome the difficulty of repeated sampling, the investigation was also aimed to collect samples at different times to find out the effect, if any, of different sampling time on the levels of gastrin and pepsinogen.

Material and methods

Plasma gastrin and pepsinogen levels were determined in sheep of Marwari breed belonging to farmers' stock of arid tract of Rajasthan state, India. The animals were maintained on the free-range grazing system. The animals, from which the blood samples collected, were grouped into healthy, haemonchus infected and drought affected. In healthy animals sampling was carried out in two patterns i.e. one time random sampling and three times sampling. In one time random sampling cases, the blood was drawn once, irrespective of time, for the preparation of plasma from 17 apparently healthy adult Marwari sheep of either sex (10 male and 7 female). The samples were collected as a part of routine health checkup during moderate ambience (maximum temperature varied between 26 and 29 °C) and animals were free from endo-parasites as assessed by routine faecal examination. In three times sampling cases blood was obtained from the same 17 adult Marwari sheep by samplings three times i.e. morning, mid-day and evening and the means were presented irrespective of sex.

In the haemonchus infected group, blood samples were obtained (from the slaughter house) from other 18 Marwari sheep of either sex (9 male and 9 female) at the time of slaughtering which had haemonchus infection detected at the time of slaughter.

In the drought affected group, 20 blood samples (10 male and 10 female) were collected from drought affected Marwari sheep of arid tract having the history of pica belonging to farmers' stock.

All the samples were collected in sterile tubes containing EDTA (di-potassium) as an anticoagulant for plasma separation. Plasma gastrin levels were determined by double antibody gastrin 125 I radioimmunoassay (RIA Kit, DPC, USA) as per the manual supplied with RIA kit in the Radio Isotope Laboratory, College of Veterinary and Animal Science, Bikaner, India. The materials supplied in the kit included gastrin antiserum, 125 I gastrin, gastrin calibrators and precipitating solution consisting of goat anti-rabbit gamma globulin and dilute polyethylene glycol in saline. In the double antibody gastrin procedure a competition was there between 125 I labeled gastrin and gastrin hormone in plasma for the sites on gastrin specific antibody. After incubation for 2 hours (15-28°C), bound was separated from free by PEG accelerated double antibody method. A foam decantation rack was used to decant the supernatant, retaining the precipitate for counting. Counting was carried out by using 125 I Gamma counter (EC, India).

Plasma pepsinogen was determined by a colorimetric method (7) with modifications (12) using bovine albumin fraction V (CDH, India) and glycine (Loba Chemie, India) to prepare substrate mixture. In the method plasma was added to a substrate mixture (0.9 % bovine albumin fraction V in glycine buffer, pH 2.0) and mixed thoroughly. An aliquot of this mixture was incubated in a water bath at 37°C for 24 hours. Then reaction was stopped by adding 4 % trichloroacetic acid (Glaxo, India) solution (12). After filtration of the protein precipitate the concentration of acid soluble tyrosine was determined by Folin Coicalteau's reagent (CDH, India). For this in each 2 ml of filtrate 0.5 ml of Folin Coicalteau's reagent and 10 ml of 0.25N sodium hydroxide (Qualigens, India) were added. Two ml of tyrosine (BDH, India) standard was also treated in the same way. The optical density was read at 680 m μ wave length using a spectrophotometer (Systronics, India). The enzyme activity was expressed as milli-units (mU) tyrosine (13).

Correlation was determined by MSTAT computer programme. Statistical significance for individual

parameter between male and female, healthy and infected, healthy and drought affected, morning and mid-day sampling, and morning and evening sampling was analysed by paired- t test (14).

Results

The mean values of plasma gastrin and pepsinogen in Marwari sheep are presented in two tables. Mean values that are presented in the table 1 belong to healthy, haemonchus infected and drought affected animals. They are further grouped into male and female animals. Non significant ($p>0.05$) difference was observed in the respective mean values of plasma gastrin and pepsinogen between male and female within each category i.e. healthy, haemonchus infected and drought affected animals.

Table 1: Plasma levels of gastrin and pepsinogen in healthy, haemonchus infected and drought affected Marwari sheep

S.No.	Groups	Gastrin (pg/ml)	Pepsinogen (mU tyrosine)
1.	Healthy		
(a)	Overall (17)	103.45± 10.41	153.61± 13.21
(b)	Male (10)	100.32±11.41	150.41± 14.11
(c)	Female (7)	106.41± 13.21	156.4± 11.61
2.	Haemonchus Infected		
(a)	Overall (18)	489.61 ±12.33 ^{bd}	772.31± 12.2 ^{bd}
(b)	Male (9)	485.45 ±13.6 ^{bd}	769.21 ±10.12 ^{bd}
(c)	Female (9)	494.32 ±11.00 ^{bd}	776.30 ±14.40 ^{bd}
3.	Drought affected		
(a)	Overall (20)	310.33± 15.1 ^{bd}	460.01± 11.5 ^{bd}
(b)	Male (10)	308.97± 16.0 ^{bd}	457.87± 12.0 ^{bd}
(c)	Female (10)	311.51± 14.9 ^{bd}	363.45± 10.3 ^{bd}

1. Figures in the parentheses indicate number of animals.

2. Healthy overall mean value of each parameter was compared with the respective mean value of infected and drought affected animals. The significant ($p<0.05$) variation has been shown by superscript 'b'.

3. The mean values of each parameter of haemonchus affected and drought affected animals were compared and means superscribed by letter 'd' within a given parameter differed significantly ($p<0.05$) from the respective means of other group.

The overall mean values of plasma gastrin and pepsinogen of haemonchus infected and drought affected animals were significantly ($p<0.05$) higher than the respective overall mean value of healthy animals. Haemonchus infected animals showed a greater rise in the mean values of both the parameters as compared to drought affected animals.

Table 2: Plasma levels of gastrin and pepsinogen in healthy Marwari sheep

S.No.	Groups	Gastrin (pg/ml)	Pepsinogen (mU tyrosine)
A.	One time random sampling Overall (17)	103.45± 10.41	153.61± 13.21
B.	Three times sampling Overall, three times sampling (51)	104.0± 10.12 ^a	152.6 ± 13.0 ^a
(a)	Morning sampling (17)	99.2± 11.0 ^{ac}	147.5±12.75 ^{ac}
(b)	Mid -day sampling (17)	105.0± 9.23 ^{ac}	152.7 ± 15.62 ^{ac}
(c)	Evening sampling (17)	109.8± 11.3 ^{ac}	159.4± 12.56 ^{ac}

1. Figures in the parentheses indicate number of observations from 17 healthy animals.

2. Overall mean value of one time random sampling of each parameter was compared with the respective mean value of overall three times sampling, morning, mid-day and evening sampling. The non-significant ($p>0.05$) variation has been shown by superscript 'a'.

3. The non-significant ($p>0.05$) variation among the mean values during morning, mid-day and evening samplings has been shown by superscript 'c'.

Mean values presented in the table 2 are of healthy animals categorised according to one time random sampling and three times sampling. Overall mean value of one time random sampling of each parameter was compared with the respective mean value of overall three times sampling, morning, mid-day and evening sampling. The non-significant ($p>0.05$) variation was observed in each case. Further in three time sampling category the mean values obtained during morning, mid-day and evening samplings were compared from each other and the variations were non-significant ($p>0.05$) among the mean values.

A significant ($p<0.05$) correlation ($r =0.921^*$) between the values of plasma gastrin and pepsinogen in haemonchus infected and drought affected sheep was observed.

Discussion

The overall mean value of plasma gastrin in the present study for healthy animals was found in the middle range of the values obtained by earlier workers in sheep (15,16) and it was more or less similar to those recorded in calves (9) and cows (17). The healthy overall mean value of plasma pepsinogen in the present study was almost comparable to those given for lambs (4) and sheep (16).

The non significant ($p>0.05$) effect of sampling time showed that feeding did not affect the levels of gastrin and pepsinogen in present study. It could be due to the fact that in ruminants the abomasum receives a continuous, though variable inflow of forestomach material (18) therefore sampling time did not affect the values of gastrin and pepsinogen. Earlier report (15) also suggests that mean plasma gastrin concentrations do not increase in response to feeding.

The pattern of changes in the values of plasma gastrin and pepsinogen was similar in the present study. The values of both the parameters increased in haemonchus infected and drought affected animals when compared to healthy animals. Similar findings were reported in camel (2). Serum gastrin concentrations were observed elevated in parasitised sheep (19). Earlier researchers (4) found an increase in plasma gastrin and pepsinogen levels in lambs infected with haemonchiasis and in sheep infected with *Ostertagia circumcincta* (6, 16).

Earlier workers have reported increased levels of serum pepsinogen in cattle with abomasal ulcers (6, 20); of plasma gastrin in cattle with bleeding abomasal ulcers (17) and of serum pepsinogen and gastrin in bovine ostertagiasis (9, 12).

A significant correlation between the values of plasma gastrin and pepsinogen in haemonchus infected and drought affected sheep (4) indicated that both the parameters can be used simultaneously or individually to assess the abomasal dysfunction. Plasma pepsinogen estimations are performed routinely in many laboratories as an aid in the diagnosis of ostertagiasis. Elevated values also occur in haemonchosis and levels decline after effective anthelmintic treatment. Plasma gastrin concentrations reflect the size of abomasal worm burden in both young and older animals (21).

Haemonchus contortus is one of the most abundant infectious agents in sheep around the world, causing great economic damage to sheep farms. Nematode larvae developing within the glands

cause local loss of parietal cells and mucous cell hyperplasia whereas reduced hydrochloric acid secretion, increased serum gastrin and pepsinogen concentrations and generalized histological changes are associated with parasites in the abomasal lumen. Parietal cells with dilated canaliculi and degenerative changes typical of necrosis are present soon after the transplantation of adult worms, and abomasal secretion is also affected (22). The massive doses of parasites produce a significant decrease in acidity and increase in sodium ion concentration of abomasal fluid. This is followed by an increase in plasma pepsinogen (23). The changes in abomasal environment are due to increased permeability of mucosa. Rise in abomasal pH is an important stimulus for gastrin release (2).

Anaerobic bacteria survive in greater numbers as the pH rises. Failure to lyse bacteria may affect adversely the nutrition of the host. The parasites may initiate the pathophysiology through the release of excretory or secretory products which either act directly on parietal cells or indirectly through enterochromaffin-like cells by provoking inflammation or by disrupting the protective mucosal defense system. Parietal cell dysfunction leads to loss of mature chief cells, mucous cell hyperplasia and hypergastrinaemia. Inflammation increases circulating pepsinogen concentrations and may also contribute to increased gastrin secretion (22).

The responses of gastrin and pepsinogen in affected Marwari sheep in the present study certainly indicated towards their diagnostic significance in abomasal dysfunctions. Probably the presence of parasites or inanimate objects in pica damaged the mucosa causing an impairment of abomasal function. Recent work in camel has demonstrated a marked increase in levels of the plasma gastrin and pepsinogen in pica affected camels which had sand in their third compartment damaging the mucosa (2). Damage to mucosa causes a drop in hydrochloric acid production (22) resulting in elevation of gastric pH which is accompanied by higher gastrin and pepsinogen release (16, 22, 24). Further the results suggested that one time random sampling can be carried out in suspected clinical cases for the determination of plasma gastrin and pepsinogen as reported by earlier workers (2). The data obtained can be used as a base line for the future studies in this direction in Marwari sheep or other breeds of sheep.

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UPORABA VREDNOSTI GASTRINA IN PEPSINOGENA V KRVNI PLAZMI ZA DIAGNOSTIČNE POKAZATELJE OKVARE SIRIŠČNIKA PRI OVCAH PASME MARVARI V SUŠNIH PODROČJIH

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Povzetek: Hormon gastrin izločajo gastrinske celice v piloričnem delu siriščnika v krvni obtok. Preko krvi gastrin prihaja do parietalnih celic in pomembno vpliva na izločanje želodčne kisline in pepsinogena, ki je proencim, neaktivna oblika pepsina, najpomembnejšega proteolitičnega encima v želodčnem soku. Da bi določili vlogo gastrina in pepsinogena pri diagnostiki zajedavskih invazij in drugih motenj siriščnika, smo določili vrednosti gastrina in pepsinogena v krvni plazmi ovc pasme marvari, ki so del čred v polpuščavskem delu zvezne države Radžastan v Indiji. Živali, pri katerih je bila odvzeta kri, smo razdelili v tri skupine: zdravo, invadirano s hemonhusi in prizadeto zaradi pomanjkanja vode. Pri zdravih živalih smo vzorce krvi jemali na dva načina: z enkratnim naključnim odvzemom ali pa trikrat zapored v istem dnevu. Skupne povprečne vrednosti gastrina in pepsinogena v plazmi zdravih ovc so bile $103,45 \pm 10,41$ pg/ml oz. $153,61 \pm 13,21$ mU tirozina. Pri obeh skupinah, prizadetih bodisi zaradi zajedavcev ali suše, smo opazili statistično značilno ($p \leq 0,05$) povečanje obeh parametrov v primerjavi z zdravimi živalmi. Čas jemanja krvi ni vplival na vrednosti gastrina in pepsinogena, kar pomeni, da tudi hranjenje ne vpliva na njune vrednosti. Ker pa se oba parametra spremenita pri prizadetih živalih, ju lahko uporabimo za diagnostična pokazatelja okvare siriščnika. Verjetno tako zajedavci kot neorganski predmeti, ki jih ovce zaužijejo med bolezenskim poželenjem po nenaravnih hrani, poškodujejo želodčno sluznico in s tem okvarijo delovanje siriščnika. Poškodba sluznice ima za posledico tudi povečano sproščanje gastrina in pepsinogena. Rezultati preiskave so tudi pokazali, da ob sumu, da gre za klinične primere, zadošča enkratni odvzem krvi ne glede na čas dneva oz. hranjenja. Pridobljeni podatki lahko služijo za osnovo pri nadaljnjih proučevanjih obremenitve ovc marvari in drugih pasem.

Ključne besede: suša; gastrin; *haemonchosis*; ovce marvari; pepsinogen; uživanje nenaravne hrane