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AMELIORATIVE EFFECTS OF DIETARY SELENIUM SUPPLEMENTATION ON HIGH-PROTEIN DIET-INDUCED RENAL ALTERATIONS IN DOMESTIC GOAT CAPRA HIRCUS

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Abstract
Eighteen cross-breed goats were equally divided in three groups such as: A=Low concentrate diet [LC], B=High concentrate diet [HC] and C=HC+Se (Se=0.3mg kg^{-1}). The goats of each group (n=6) were fed these diets for 10 weeks period and subsequent ameliorative effects of dietary Se on high-protein diet-induced renal changes were investigated. Significant treatment effect ($p<0.05$) can be perceived from the data of groups A, B and C from kidney weight (31.61, 35.37, 40.32 g); length (5.75, 6.44, 6.48 cm); width (3.28, 3.93, 4.54 cm); and thickness (2.07, 2.39, 2.76 cm), respectively. The apparent changes in histological traits in renal cortex were also significant ($p<0.05$) in groups A, B, C for density of renal corpuscles (9.12, 9.69, 11.76); diameter of Bowman's space (112.65, 107.02, 92.37 µm); Cros-sectional area (CSA) of glomerulus (898, 925, 1086.6 µm); SCA of proximal convoluted tubules (71.21, 75.48, 84.74 µm); SCA of distal convoluted tubules (36.03, 40.35, 50.8 µm); CSA of collecting tubules (66.07, 68.05, 76.64 µm); CSA of distal tubules (45.68, 47.97, 53.91 µm) and CSA of thin tubules (19.46, 23.42, 27.44 µm), respectively. The histopathological examination of kidney indicated that kidneys of goat in group A and B (without Se), showed observable regions of tubular atrophy and interstitial fibrosis; while group-C (HC+Se) has a significant protective effect. Histomorphological examination of left kidney medulla showed that in LC diet (A), the kidney tissue had slight negative effects due to oxidative stress; while kidney of group-B (HC diet without Se) showed some signs of stress and damage. However, the kidney of group-C goat (Se-rich HC diet) revealed marked protective and curative effects on renal structure. The epithelial structure in LC group appears to be rather normal; while HC group exhibits significant inflammatory cell infiltration, goblet cell depletion and epithelial injury as well as some minor histological abnormalities. However, in HC+Se group, there was a visible decrease in histological damage, as seen by less epithelial injury, more intact goblet cells and less infiltration of inflammatory cells

INTRODUCTION

The rural economy of Pakistan has considerable reliance on livestock farming and in this goat goat farming is crucial for meat, milk and hides. With goat population of 80 million (GoP, 2024), Pakistan is third largest goat producer of the world after China and India. The most important pre-requisition for efficient goat production is to optimize good animal health. Selenium (Se) is essential trace element required for maintenance of many physiological activities in human and animal.

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In order to keep continuity in their production they must require exogenously supply of minerals trace elements where the Se provides protective role via the specific proteins. It is an integral component of seleno-proteins such as glutathione peroxidase, which makes an important part of anti-oxidation system in animal body (Fairweather-Tait et al., 2010). Being trace mineral Se is composed of some seleno-proteins and various enzymes including glutathione peroxides (GSH-Px), which possess some antioxidant characteristics (Mielczarek et al., 2018). Dietary Se supplementation within the ruminants is practiced to avoid the risk of its deficiency in animals, particularly in regions where the soils are Se deficient. Organic Se has antioxidant, anti-inflammatory and chemo-protective properties (Pappas et al., 2008). Even minute fluctuations in Se level in diet has many significant impacts on animal health and performance (Omeje et al., 2016). Goat and sheep growth performance is directly correlated with selenium and selenioproteins. Sufficient amounts of selenium facilitate metabolic functions, improve feed efficiency, and encourage the growth of muscle. To maximise development potential in small ruminants, seleniumproteins such GPx and TrxR aid in lowering metabolic stress throughout growth stages (Pappas et al., 2017). Studies have indicated that supplementing with selenium enhances weight increase, feed conversion efficiency, and carcas quality in sheep and goats (Mehdi & Dufrasne, 2016). Furthermore, because increased oxidative stability inhibits lipid peroxidation in muscle tissues, diets high in selenium have been associated with higher-quality beef (Schröder et al., 2019).

The protein requirement in goat varies depending on the age, physiological state and productivity of the animal. High-protein diets are formulated to meet these increased demands, especially in lactating and growing goats (NRC, 2007). However, excessive protein intake can lead to metabolic imbalances and health issues, including renal stress (Gupta et al., 2015). As goats are ruminants commonly raised for meat, milk, fiber; and their dietary needs are crucial for optimal health and productivity (Zhou et al., 2016). High-protein diets are often employed to enhance growth and milk production, but such diets can impose significant stress on the kidneys, leading to potential renal dysfunction (Singh et al., 2018). These quality diets increase the production of urea, leading to a higher renal workload. However, kidneys are responsible for filtering blood, excreting waste products, and maintaining fluid and electrolyte balance. Moreover, these diets elevate the production of nitrogenous waste, primarily urea, which the kidneys must excrete (Zhou et al., 2016); this increased workload can lead to renal hypertrophy and renal damage over time (Zhang et al., 2019).

Kidneys are main organs of excretion in mammals including ruminants, which play an important position in continuation of homeostasis. To counteract the effect of oxidants (free radicals), kidneys contain endogenously produced antioxidants enzymes produced by proximal and distal tubules of the nephrons and from the specific regions of malphigian body where the specialized cells known as Maculladensa which are generally responsible to provide emeleorative effect by secreting glutathione peroxidase (Schomburg et al., 2011). The synthesis and activity of GSH-Px is correlated with the availability of Se in kidney (Köhrle et al., 2005). Unlike the other organs such as liver, ovaries, pituitary gland etc in which the tissue Se concentration is decreased if animals fed poor Se diet, the kidne situations (Shi et al., 2011). HC diet-induced oxidative stress causes gut epithelial damages associated with apoptosis; whereas, the addition of Se in HC diet alleviated such damaging changes (Samo et al., 2020). Zhang et al. (2014) indicated that Se supplementation in goats led to a downregulation of pro-apoptotic genes; this shift suggests a reduction in apoptosis, thereby promoting cell survival and renal integrity (Schomburg et al., 2011). Se effect on urine analysis, renal function, creatinine, and electrolyte levels in goats emphasized positive role of Se in renal health. Zhou et al. (2017) reported that Se supplementation in goats was associated with significant improvements in renal function markers, including reduced serum creatinine and BUN levels, suggesting enhanced glomerular filtration rate (GFR) and reduced renal stress. Antioxidant properties of Se help counteract oxidative stress in renal tissues, reducing cellular damage, crucial for preserving renal function (Zhang et al., 2018).

Reactive oxygen species (ROS) detoxification is the main mechanism by which selenium proteins

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contribute to antioxidant defence. Glutathione peroxidase (GPx), one of the most researched selenoproteins, guards against oxidative damage to cells by lowering hydrogen peroxide and organic hydroperoxides (Pappas et al., 2017). GPx activity is essential in reducing oxidative stress in sheep and goats, which can be made worse by environmental variables including heat stress, parasite diseases, or inadequate diet (Sordillo, 2013). It has been demonstrated that supplementing with selenium increases GPx activity, lowering oxidative stress and enhancing general health in small ruminants (Kaur et al., 2021). According to research, selenium supplementation, for instance, increases GPx activity in sheep and reduces oxidative stress indicators in their tissues (Aziz et al., 2023).

Renal corpuscles, convoluted tubules, and a dense capillary network make up the kidney histologically. The glomerulus and Bowman's capsule comprise the renal corpuscles, which are mostly located in the cortex. Reabsorption is made easier by the cuboidal epithelial cells with microvilli lining the proximal convoluted tubule (PCT). According to Ali et al. (2023), goat PCTs have more densely packed microvilli than other ruminants, which is an adaption for effective nutrition reabsorption. Changes in Se levels have been associated with alterations in several selenoproteins (Reddi & Bollinen, 2001). The best selenium supplementation has been shown in several studies to improve antioxidant capacity of goat, which is essential for reducing kidney damage during oxidative stress conditions like infections or toxic exposures (El-Shaer et al., 2023). This improves renal function in goats. Further research has revealed that selenium controls the process of kidney cell death, which helps to preserve renal structure and function (Ali et al., 2022). The nephrons are responsible for filtration, excretion and reabsoption, and they regulate ion balance, water content, help to stabilise blood pressure. This regulation of ion balance and water content (Osmoregulation) of the blood plasma means that regulates all the other body fluids (Vosough & Mozaffari, 2009).

Kidneys produce large ROS concentrations during oxidative metabolism, that should have to remove simultaneously to reduce oxidative damage. Intezar (2018) revealed significant ($p<0.05$) increase in overall weight, length, width, and volume; whereas other morphological features like thickness, longitudinal circumference and cross circumference changed significantly ($p<0.05$). There was significant ($P<0.05$) increase epithelium height of bowmans capsule with Se supplementation, but no effect of Se on thickness of Glomerulus. Antioxidant values were increased with Se supplementation significantly (Awais, 2017). Most of the previous studies regarding with histology and tissue antioxidant status have been conducted as antioxidant effects of Se in liver spleen and other organs (Reddi & Bollinen, 2001). However, restricted of very fewer studies have been conducted on kidney that mainly focused on primates while the ruminants have been given much lesser concentration. This study was aimed at investigating the ameliorative effects of dietary Se on high-protein diet-induced renal changes and alteration in morphology of kidney in goat.

MATERIALS AND METHODS

1. Methodology

1.1 Animal Selection and Adaptation

Eighteen cross-breed goat kids of approximately 3-4 months of age, weighing 10-13 kg body weight were randomly selected for study. The experimental animals were purchased from nearby small-scale goats farm and brought to the Livestock Experimental Station, Faculty of Animal Husbandary and Veterinary Sciences, Sindh Agriculture University, Tandojam. Two week before the start of experiment, goat kids were offered free access to the diet containing a forage and concentrate feed, to ensure the adaptation to the diet. During adaptation period, all the animal were ear tagged for identification, drenched for deworming and injected with ivermectin againist helminthes and other parasitic insects, and vaccinated against some common infectious diseases.

1.2 Experimental design and feeding management:

Groups	Treatments	Treatment description

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LC	Control	Green fodder
HC	Concentrate (30 g/kg BW/day)	Green fodder + Concentrate
HC+Se	Concentrate (30 g/kg BW/day) + Se (0.5 mg/kg Feed)	Green fodder + Concentrate + Selenium

1.3 Slaughtering, sample collection and measurements:

At the end of experimental period, the goat kids were slaughtered, the abdomen was opened, and both the right and left kidneys were removed carefully. Immediately after removal of kidneys, their extra fat was rinsed, washed with cold buffer solution and then the gross morphology and biomorphometry was carried out. After completion of biomorphometry, two tissue samples were taken from kidneys; first tissue sample was collected for measurement of antioxidants and another tissue sample was collected in 4% paraformaldehyde solution for conducting histomorphology.

1.4 Biomorphometry of kidneys:

The biomorphometrical parameters of kidneys included kidney weight, kidney length, kidney width, kidney circumference and kidney volume, kidney diameter of cortex and medulla of kidneys.

1.5 Histomorphology of kidney:

The initial phase in the investigation of the histomorphology of ruminant kidneys was the comprehensive collection of kidney samples, either immediately following sacrifice or after death. Phosphate-buffered saline (PBS) was employed to cleanse the kidneys of blood and surface contaminants. In order to preserve their structural integrity, the renal pelvis, cortex, and medulla were separated from the kidney and preserved in 10% neutral-buffered formalin for a minimum of two days. After fixation, the samples were dehydrated using a graduated series of ethanol (ranging from 70% to 100% alcohol). Finally, they were embedded in paraffin wax blocks for sectioning and cleansed with xylene to remove alcohol. Thin segments, typically 4-6 µm thick,were cut using a rotary microtome and subsequently affixed to adhesive-coated glass slides to ensure their stability during staining. The mounted slices were deparaffinized in xylene, rehydrated with ethanol concentrations that decrease, and stained with haematoxylin and eosin (H&E) to identify nuclei and cytoplasmic components.

Periodic Acid-Schiff (PAS) was employed in specialised examinations for basement membranes, while Masson's Trichrome was used for fibrosis and collagen. The stained sections were analysed for abnormalities in the renal histological characteristics, including the glomeruli, renal tubules, loops of Henle, interstitial spaces, and blood arteries, using a light microscope. These abnormalities may include necrosis, inflammation, fibrosis, or apoptosis. In order to acquire high-resolution photographs, the microscope was equipped with a digital camera. Morphometric software, such as ImageJ, was then employed to analyse these images. Measurements such as interstitial thickness, tubular diameter, and glomerular dimensions were documented. Using this technology, the structural integrity and pathological alterations of goat kidneys that have undergone a variety of physiological or experimental interventions can be thoroughly examined. The morphometric procedures were carried out with a computerized image analysis program (IPP, Image-Pro plus Version 6.0, Media Cybernetic Inc) on Hematoxylin and Eosin-stained tissue sections.

1.6 Development of kidney size using ultrasonography in goat

Two goat kids from each treatment group were selected for performing ultrasonography; the ultrasonography was performed at the Department of Veterinary Surgery, Faculty of Animal Husbandry & Veterinary Sciences, Sindh Agriculture University Tandojam. A portable ultrasound device featuring a linear transducer (5–7.5 MHz) was used. The transducer maintains optimal contact with the skin following the shaving of the lumbar region, precisely associated with the anatomical position of the kidney; while the goats were gently restrained to reduce stress levels. To improve the clarity of the image and reduce air interference, a coupling agent was utilised. The transducer was strategically placed either transabdominally or transrectally, depending on the size of the animal and the specific location of the kidney. The kidneys appeared on the ultrasound

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screen as bean-shaped entities, showing clearly the cortical and medullary regions. The longitudinal and transverse planes serve as essential references for quantifying the dimensions of the kidney, encompassing its length, breadth, and thickness. Measurements of these dimensions were captured and analysed in real-time to determine kidney size. Ultrasonography serves as a non-invasive, reproducible, and precise technique for assessing kidney dimensions and detecting abnormalities such as hydronephrosis, nephrolithiasis, and changes induced by dietary factors or disease processes.

The assessment of renal physiological function involves centrifuging whole blood at 3000 rpm for a duration of 10 minutes, facilitating the separation of plasma or serum from the cellular components of the blood. In realm of laboratory analysis, spectrophotometers and fully automated biochemistry analysers served as essential tools for quantifying biochemical parameters, including creatinine, electrolytes such as sodium, potassium, and chloride, as well as blood urea nitrogen (BUN). These metrics illustrated the efficiency of renal filtration processes and maintenance of homeostasis in water and electrolyte balance. A comprehensive assessment of renal function and hydration status includes measurement of plasma osmolality alongside specific protein levels, including albumin and globulin.

To assess physiological parameters, ultrasonography was employed to examine the structural integrity of the kidneys. Ultrasonographic imaging effectively captures kidney dimensions, as well as the echogenic characteristics of both the cortex and medulla, while also identifying any abnormalities such as cysts, enlargement, or atrophy of the kidneys. Assessing urine output and conducting analyses of urine samples for parameters such as pH, specific gravity, protein and glucose. The data thus achieved were statistically analysed to discriminate the treatment effect.

RESULTS AND DISCUSSION

2. Biomorphometrical examination

2.1. Weight of kidney

Fig 1 illustrates the mean kidney weight of goats between treatments, Low concentrate (LC), HC (High Concentrate) and HC+Se (High Concentrate + Selenium), emphasizes the advantageous function of selenium supplementation. The average kidney weight of the goats in the HC+Se group was 40.32 g, which was the highest, indicating that the kidneys could effectively adjust to the demand of diet high in concentrates when Se was added. Although the high-protein diet in the goats in the HC group caused their kidney weight to increase to 35.37 g, the additional increase in HC+Se group indicates that Se may support improved renal function, assisting the kidneys in coping with the increased metabolic load and oxidative stress caused by high-protein diet.

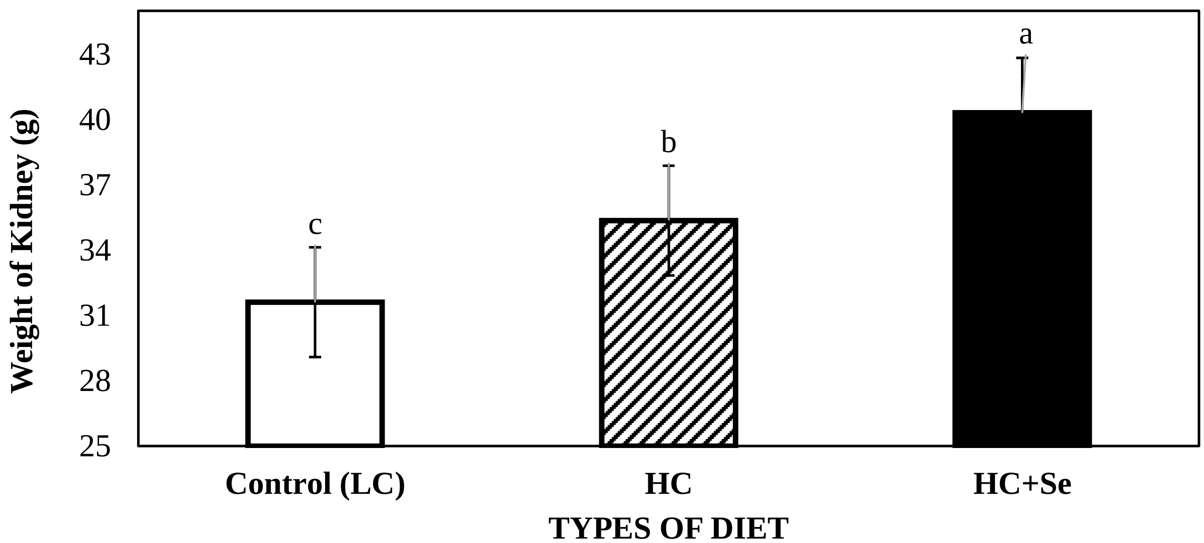

Fig 1 Weight of kidney in goats fed on HC (High concentrate) group B (n=6) and HC+Se (High concentrate + Se) group C (n=6) diet was compared to the goats fed LC (Low concentrate) group A (n=6)

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diet. Goats in various groups were fed LC, HC and HC + Se diets for a period of 10 weeks. The mean values of weight of kidney differed significantly ($p<0.05$) among all the three groups.

2.2. Length of kidney

Fig 2 displays the average kidney length of goats fed on different dietary treatments: LC, HC and HC+Se (Se 0.3 mg/kg feed). In every group, the kidneys eventually get longer. Goats given LC diet had an average kidney length of 5.75 cm, which is representative of a baseline under normal, low-concentrate feeding environment. In contrast, the HC group goat's kidney length increased marginally to 6.44 cm. The increased metabolic load and physiological alterations brought on by a high-protein diet are most likely the causes of this. The kidney length in the HC+Se group grew to 6.48 cm, suggesting that the stress of a high-concentrate diet may have been able to maintain renal function and structural integrity with Se supplementation.

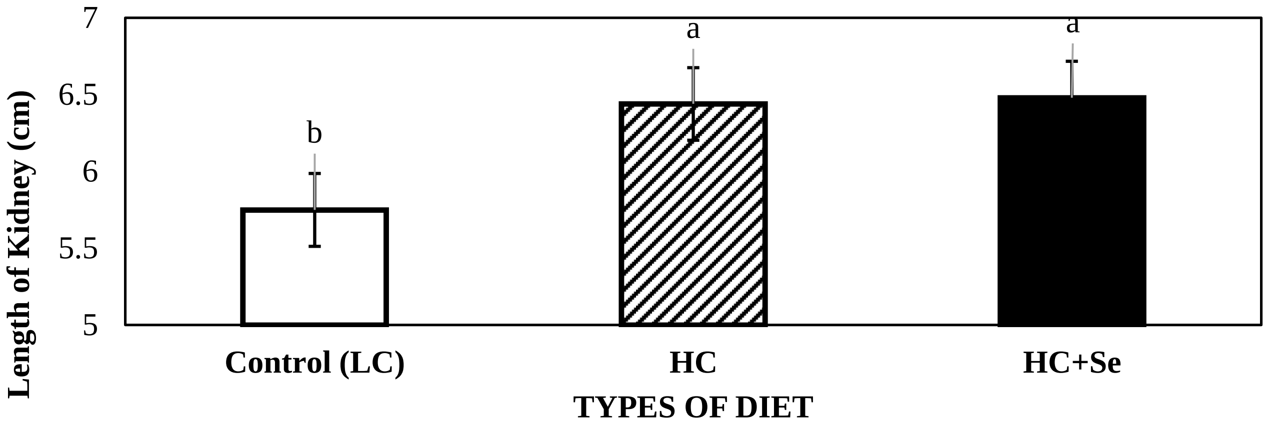

Fig 2 Length of kidney in goats fed on HC group B (n=6) and HC+Se group C (n=6) diet was compared to the goats fed LC group A (n=6) diet. Goats in various groups were fed LC, HC and HC + Se diets for a period of 10 weeks. The mean values of width of kidney differed significantly ($p<0.05$) when HC and HC+Se groups were compared to LC group; while difference between HC and HC+Se groups were nonsignificant ($P>0.05$).

2.3. Width of kidney

Fig 3 describes that the kidney widths of the LC, HC and HC+Se (Se 0.3 mg/kg) treatments significantly increased (p = <0.05). The goats in the Control (LC) group had the least kidneys width, measuring 3.28 cm, which is indicative of normal kidney size when fed a diet low in concentrates. The HC group showed an increase upto 3.93 cm, most likely as a result of renal enlargement brought on by the high protein consumption. With a kidney width of 4.54 cm, the HC+Se group displayed the maximum kidney width, suggesting that Se addition protected and adjusted kidneys to meet metabolic needs of a HC diet.

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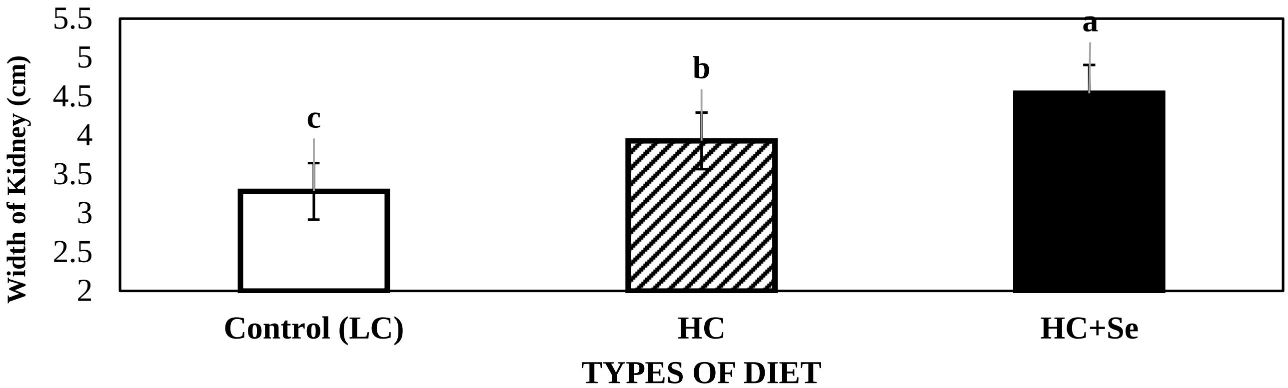

Fig 3 Kidney width in goats fed HC (High concentrate) group B (n=6) and HC+Se group C (n=6) diet was compared to the goats fed LC (Low concentrate) group A (n=6) diet. The mean values of width of kidney in goats fed Se in addition to HC diet improved significantly ($p<0.05$) over HC and LC diets.

2.4. Thickness of kidney

In comparison to LC, HC and HC+Se dietary regimens, the graph shows a substantial increase in kidney thickness ($p = 0.000$). As baseline kidney morphology under a low-concentrate diet, goats in LC group thickness was least (2.07 cm). Likely to renal stress and hypertrophy brought on by high protein intake, kidney thickness rose to 2.39 cm in HC group. SE addition may have further improved kidney development and adaptability to the high-protein diet, since the HC+Se group had the largest kidney thickness (2.76 cm).

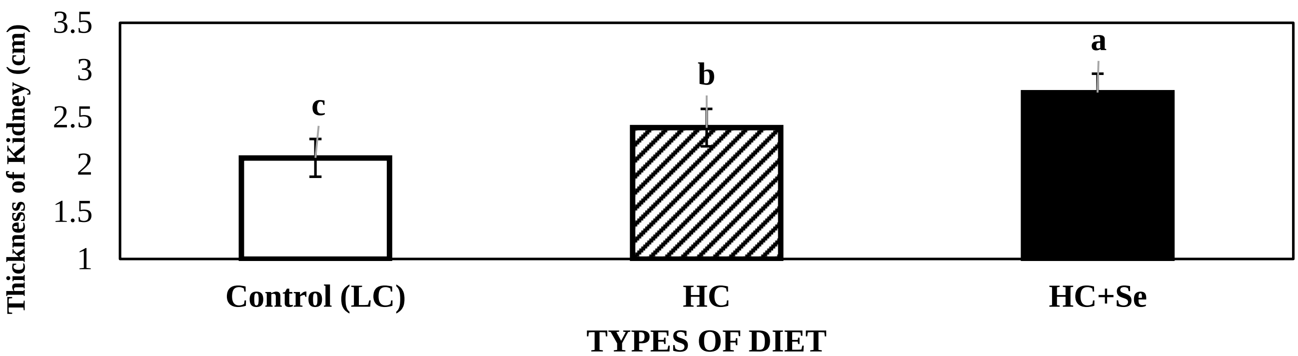

Fig 4: Thickness of kidney in goats fed on HC (High concentrate) group B (n=6) and HC+Se (High concentrate + Se) group C (n=6) diet was compared to the goats fed LC (Low concentrate) group A (n=6) diet. The mean values of thickness of kidney in goats fed HC+Se diet showed significant ($p<0.05$) improvement as compared to the goats of HC and LC groups of goats.

3. Histological characteristics in renal cortex

3.1. Density of renal corpuscles

The density of renal corpuscles in the kidneys of goats receiving three different dietary treatments, Control (LC), HC (High Concentrate), and HC+Se (Se 0.3 mg/kg), shows a significant difference ($p=0.0245$) in the graph. With 9.12 corpuscles/mm^2, the goats in the Control (LC) group exhibited the lowest density of renal corpuscles, which is indicative of baseline kidney structure on a typical low-concentrate diet. The density in the HC group marginally rose to 9.69 corpuscles/mm^2, most likely

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as an adaptive reaction to the high protein diet. With a density of 11.76 corpuscles/mm², the HC+Se group showed a considerably higher density.

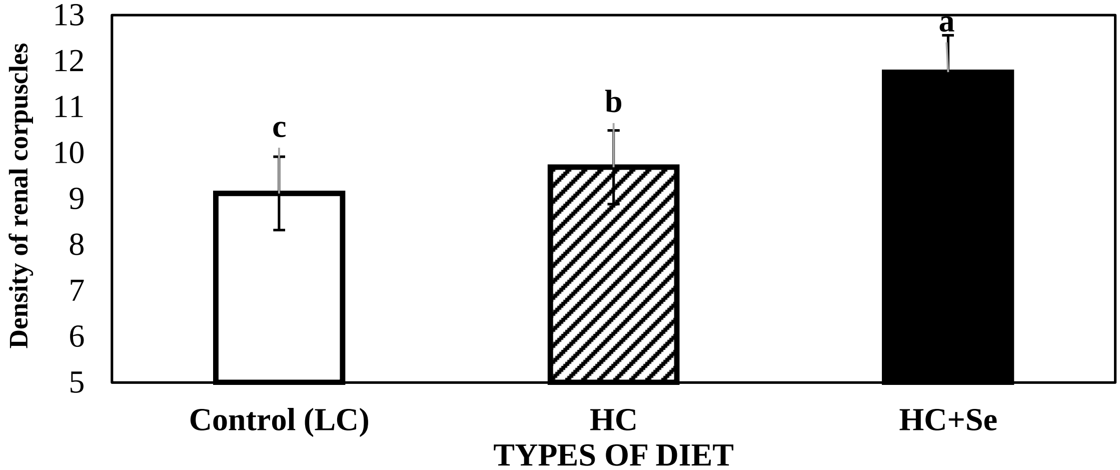

Fig 5 Density of renal corpuscles of kidney in goats fed on HC (High concentrate) group B (n=6) and HC+Se (High concentrate + Se) group C (n=6) diet was compared to the goats fed LC (Low concentrate) group A (n=6) diet. The graph suggests that Se supplementation improved proliferation and function of renal corpuscles, supporting better kidney health and adaptability to metabolic load of a high-protein diet. The beneficial effect of Se on renal corpuscle density is demonstrated by the statistically significant p-value ($p<0.05$).

3.2. Diameter of Bowman's space (µm)

The dimension of Bowman's space in the kidneys of goats given three distinct dietary treatments: Control (LC), HC (High Concentrate), and HC+Se (High Concentrate with Se at 0.3 mg/kg feed); shows significant variations ($p=0.0049$). Under a diet low in concentrate, the goats in the Control (LC) group had the highest average diameter of Bowman's space, measuring 112.65 µm. This suggests that their kidney morphology was normal. On the other hand, the HC group decreased diameter of 107.2 µm indicates that the high-protein diet may have a role in nephron structural alterations that have resulted in Bowman's space constriction. With a diameter of 92.37 µm, HC+Se group had lowest diameter, suggesting that Se supplementation further decreased the size of Bowman's gap.

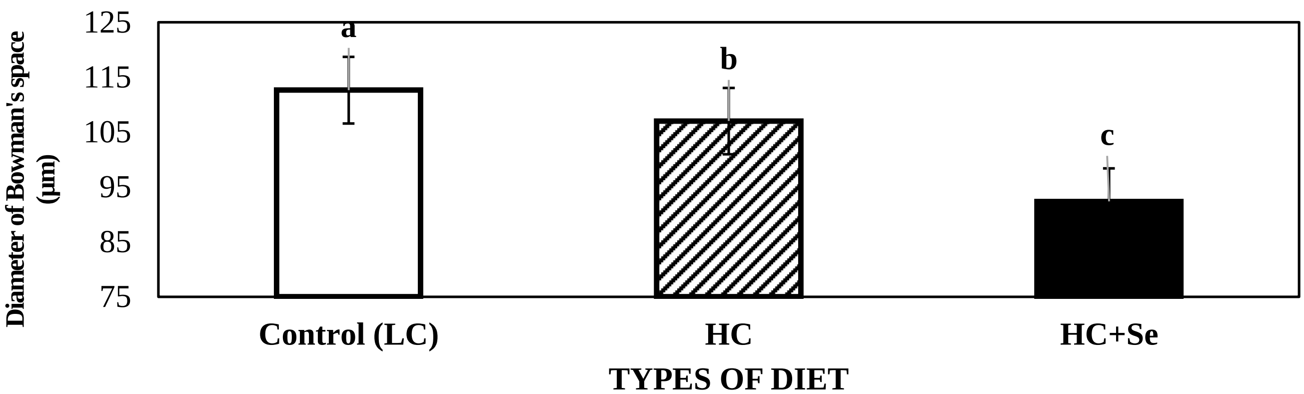

Fig 6 Diameter of Bowman's space of kidney in goats fed on HC (High concentrate) group B (n=6) and HC+Se (High concentrate + Se) group C (n=6) diet was compared to the goats fed LC (Low concentrate) group A (n=6) diet. The graph suggesting that Se supplementation further decreased the size of Bowman's gap. This might be due to changes in kidney function or an adaptive reaction to a high

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protein diet. The shape of Bowman's space in goat kidneys is influenced by dietary interventions, as indicated by the significant p-value ($p<0.05$).

3.3. C.S. Area of glomerulus (µm)

The cross-sectional area of the glomeruli in the kidneys of goats fed three different diets, HC (High Concentrate), HC+Se (High Concentrate with Se at 0.3 mg/kg feed), and Control (LC), is displayed on the graph. As a benchmark for normal kidney anatomy, the Control (LC) group measured a glomerular area of 898.0 µm². An increase of 925.0 µm² on average was seen in the HC group, indicating some degree of renal adaptation to the high-protein diet. In the HC+Se group, the largest alteration was observed in the glomerular area, which increased to 1086.6 µm². This significant rise suggests that supplementing with Se not only supports kidney function but may also encourage glomerular hypertrophy, which improves the kidney capacity to filter blood efficiently.

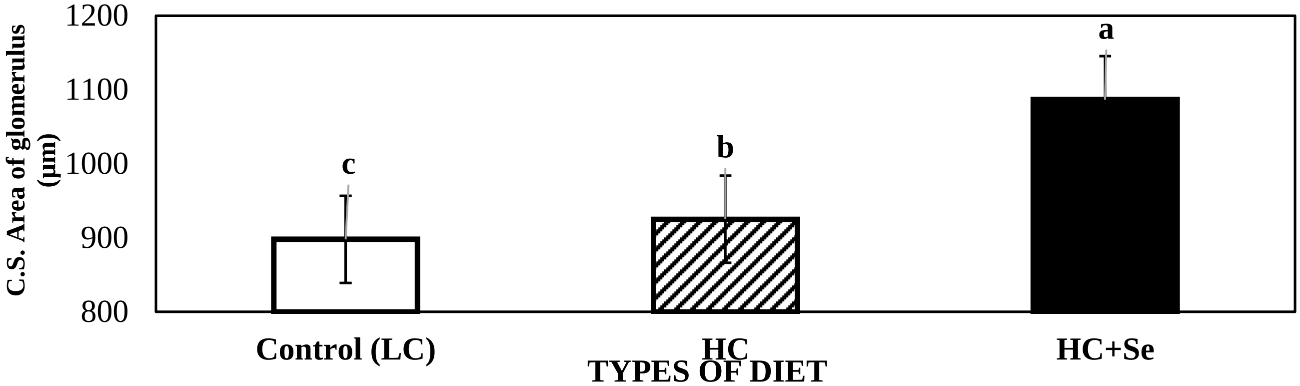

Fig 7 Cross-sectional area of glomerulus (µm) of kidney in goats fed on HC group B (n=6) and HC+Se group C (n=6) diet was compared to the goats fed LC group A (n=6) diet. The significant influence of dietary intervention on glomerular shape is shown by the noteworthy p-value of 0.0136, which also emphasizes the beneficial function of Se in maintaining renal health in high-protein environments.

3.4. Cross-sectional area of the proximal convoluted tubules

Goats were given three different dietary treatments: Control (LC), HC (High Concentrate), and HC+Se (High Concentrate with Se at 0.3 mg/kg feed). The graph displays changes in the cross-sectional area of the proximal convoluted tubules (PCT) in the kidneys of these goats. The group that had a low-concentrate diet, known as the control

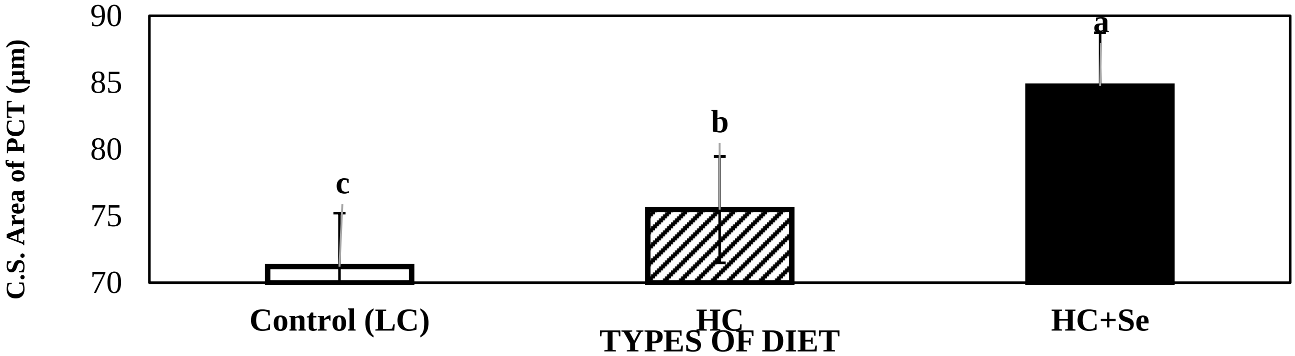

Fig 8 Cross-sectional area of the proximal convoluted tubules (CSA-PCT) of kidney in goats fed on HC (High concentrate) group B (n=6) and HC+Se (High concentrate + Se) group C (n=6) diet was compared to the goats fed LC (Low concentrate) group A (n=6) diet. The impact of nutritional interventions on the structural adaptability of PCT is demonstrated by the substantial p-value ($p<0.05$), with Se being

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particularly helpful in enhancing renal function. (LC) group, had the lowest PCT area, measuring 71.21 µm². As a result of kidney adaptation to the demands of a high-protein diet, there was a slight rise to 75.48 µm² in the HC group. Se supplementation considerably improves tubular structure, possibly boosting renal reabsorption capacity under dietary stress. The most notable improvement was seen in the HC+Se group, where the PCT area expanded to 84.74 µm².

3.5. Cross-sectional area of the distal convoluted tubules

The graph shows variations in the distal convoluted tubules (DCT) cross-sectional area in the kidneys of goats fed three different dietary treatments: control (LC), high concentrate (HC), and high concentrate with Se (HC+Se; 0.3 mg/kg feed). With a DCT size of 36.03 µm², the goats in the Control (LC) group exhibited the lowest DCT, which is indicative of normal tube construction under a low-concentrate diet. The DCT area rose to 40.35 µm² in the HC group, suggesting structural expansion as a result of the high-protein diet. The results indicate that tubular shape is strongly supported and enhanced by Se supplementation, since the HC+Se group demonstrated the highest DCT area, measuring 50.80 µm².

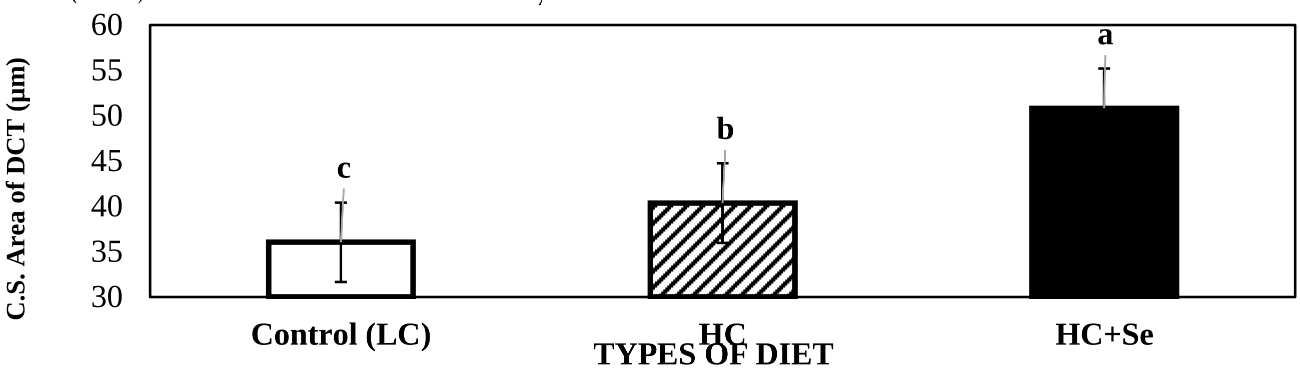

Fig 9 Cross-sectional area of the distal convoluted tubules (DCT) of kidney in goats fed on HC group B (n=6) and HC+Se (High concentrate + Se) group C (n=6) diet was compared to the goats fed LC (Low concentrate) group A (n=6) diet. The effectiveness of renal filtration and reabsorption is probably enhanced by this increase in DCT size. The various dietary treatments had a considerable influence on the DCT, as evidenced by the very significant p-value (p=0.000). Se supplementation had the most notable favourable effect on kidney structure.

3.6. Cross-sectional area of the collecting tubules

The cross-sectional area of the collecting tubules in the kidneys of goats treated with three different diets: Control (LC), HC (High Concentrate), and HC+Se (High Concentrate with Se at 0.3 mg/kg feed), is shown in the graph. As the baseline structure with a low-concentrate diet, the Control (LC) group had the lowest collecting tubule area, measuring 66.07 µm². The area rose to 68.05 µm² in the HC group, indicating a modest expansion that may be the result of a minor structural adjustment brought on by the high protein consumption. The group that supplemented with Se showed the greatest increase in collecting tubule area, measuring 76.64 µm².

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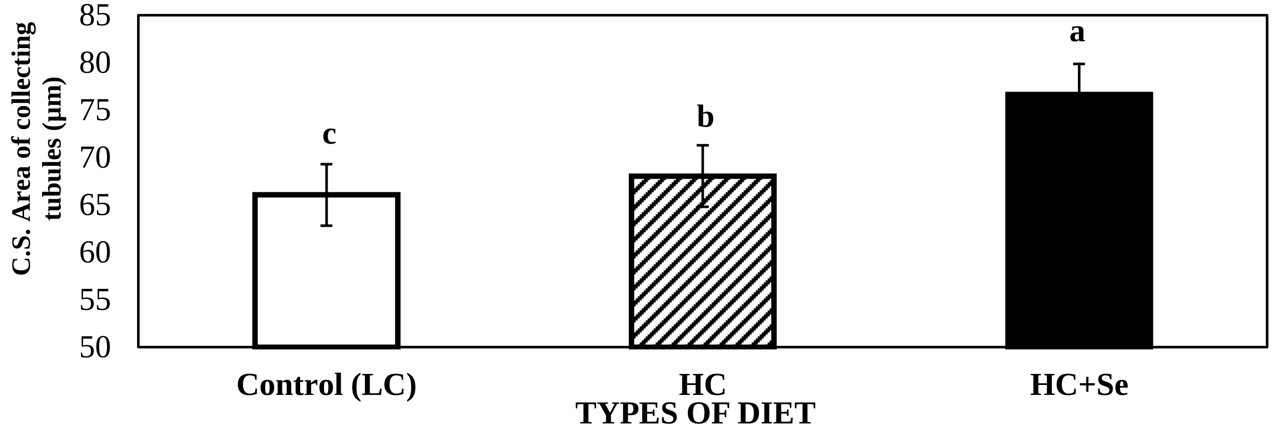

Fig 10 Cross-sectional area of the collecting tubules of kidney in goats fed on HC (High concentrate) group B (n=6) and HC+Se (High concentrate + Se) group C (n=6) diet was compared to the goats fed LC (Low concentrate) group A (n=6) diet. Better renal function is probably facilitated by this increase, which also helps with urine excretion and focus. The considerable impact of these treatments is confirmed by the p-value of 0.0281, which emphasises the importance of Se in improving the morphology of collecting tubules in particular.

3.7. Cross-sectional area of the distal tubules

The graph displays significant variations in the distal tubule cross-sectional area in the kidneys of goats fed different diets: Control (LC), HC (High Concentrate), and HC+Se (High Concentrate supplemented with Se at 0.3 mg/kgfeed). The smallest distal tubule area, measured 45.68 µm² in the Control (LC) group, reflected the normal shape under a low-concentrate diet. The HC-group showed a modest rise to 47.97 µm², suggesting a moderate reaction to increased protein consumption. With an area of 53.91 µm², the HC+Se group showed the largest expansion.

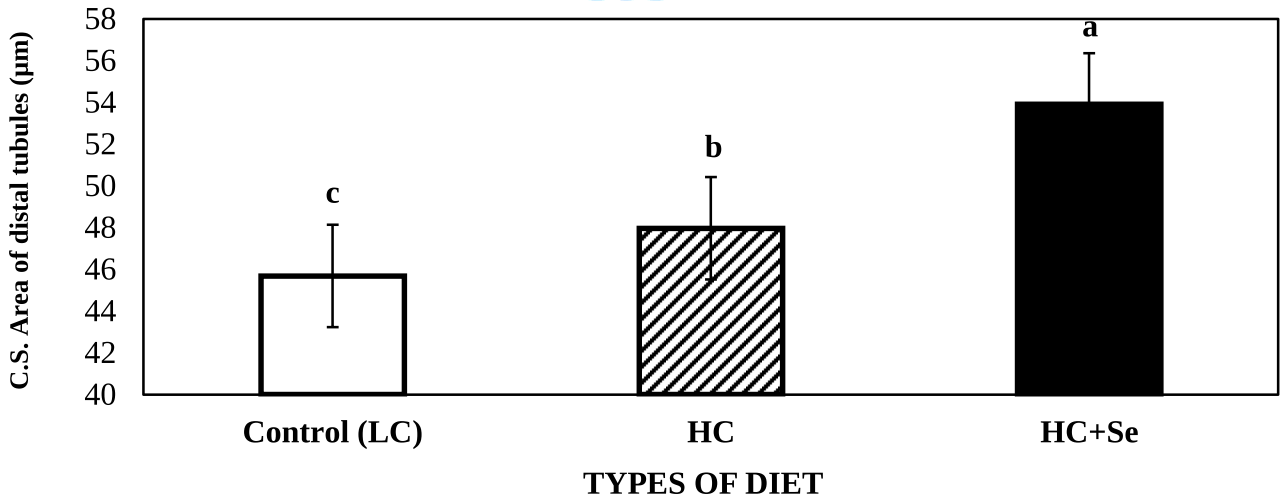

Fig 11 Cross-sectional area of the distal tubules of kidney in goats fed on HC (High concentrate) group B (n=6) and HC+Se (High concentrate + Se) group C (n=6) diet was compared to the goats fed LC (Low concentrate) group A (n=6) diet. The Se has a beneficial effect on tubular size and may even improve renal function. The significant impact of these treatments is shown by the substantial p-value (p=0.0013), wherein Se supplementation leads to increased structural adaptability of the distal tubules under dietary stress.

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3.8. Cross-sectional area of thin tubules

Significant variations in the cross-sectional area of the thin tubules in the kidneys of goats fed three different diets, Control (LC), HC (High Concentrate), and HC+Se (High Concentrate with Se at 0.3 mg/kg feed) are shown in the graph. With a narrow tubule area of 19.46 μm^2, the goats in the Control (LC) group had the shortest tubule area, which served as the baseline under low-concentrate diet conditions. A significant increase in area to 23.42 μm^2 was seen in the HC group, indicating structural modifications in response to the high-protein diet. With a important extension to 27.44 μm^2, the HC+Se group had the biggest area, suggesting that Se supplementation has a strong effect on increasing the size of the thin tubules.

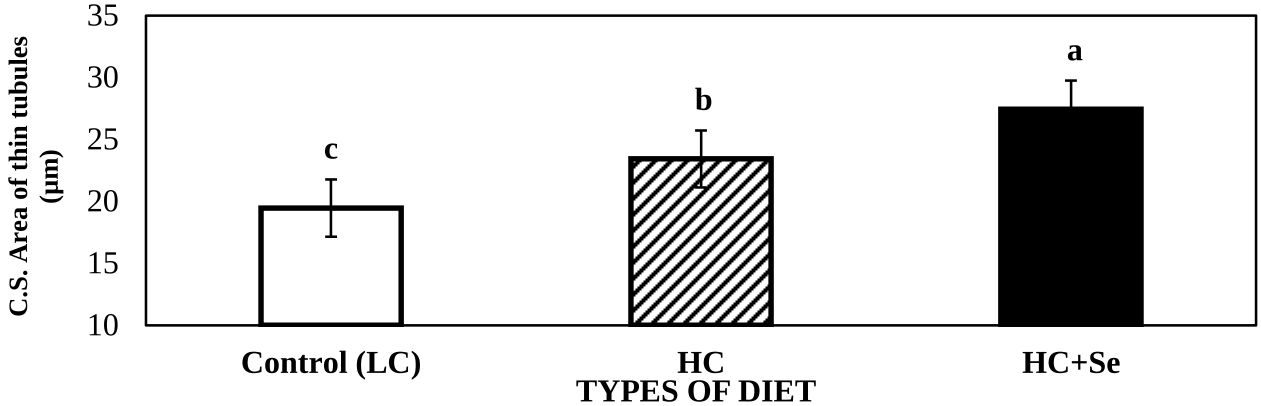

Fig 12: Cross-sectional area of thin tubules of kidney in goats fed on HC (High concentrate) group B (n=6) and HC+Se (High concentrate + Se) group C (n=6) diet was compared to the goats fed LC (Low concentrate) group A (n=6) diet. The graphical illustration of cross-sectional area of thin tubules revealed that better renal function, including improved tubular transport and filtration, is probably supported by this expansion. The major impact of these dietary therapies is shown by the extremely significant p-value ($p=0.0000$), wherein Se is found to have a key role in tubular development and adaptability.

3.9. Histopathological examination of kidney

The effect of dietary Se on the pathological characteristics in the goat kidney has been shown in histology pictures (Fig 13). The kidneys of the Control group (A), which did not receive a Se supplement, show observable regions of tubular atrophy and interstitial fibrosis. These alterations imply that kidney structures were harmed by the absence of preventive measures, which resulted in tissue scarring and decreased function. The group-B High Concentrate (HC) diet without Se exhibited more severe indications of tubular atrophy and fibrosis. The degree of damage is higher than in the Control group, suggesting that kidney degeneration is made worse by a high-concentrate diet deficient in Se, perhaps as a result of oxidative stress and inflammation. On the other hand, the group (C) that received supplemental Se has a noteworthy protective effect. Although there is a blockage of polyvinyl alcohol particles in the interlobar artery, the degree of tissue injury observed in groups A and B does not appear to be reflected in this embolus. Due to its antioxidant qualities, Se seems to protect kidneys from harm by lowering oxidative stress, which helps the kidneys function normally even while eating a diet heavy in concentrated foods. This demonstrates how Se protects kidney health by decreasing fibrosis and atrophy.

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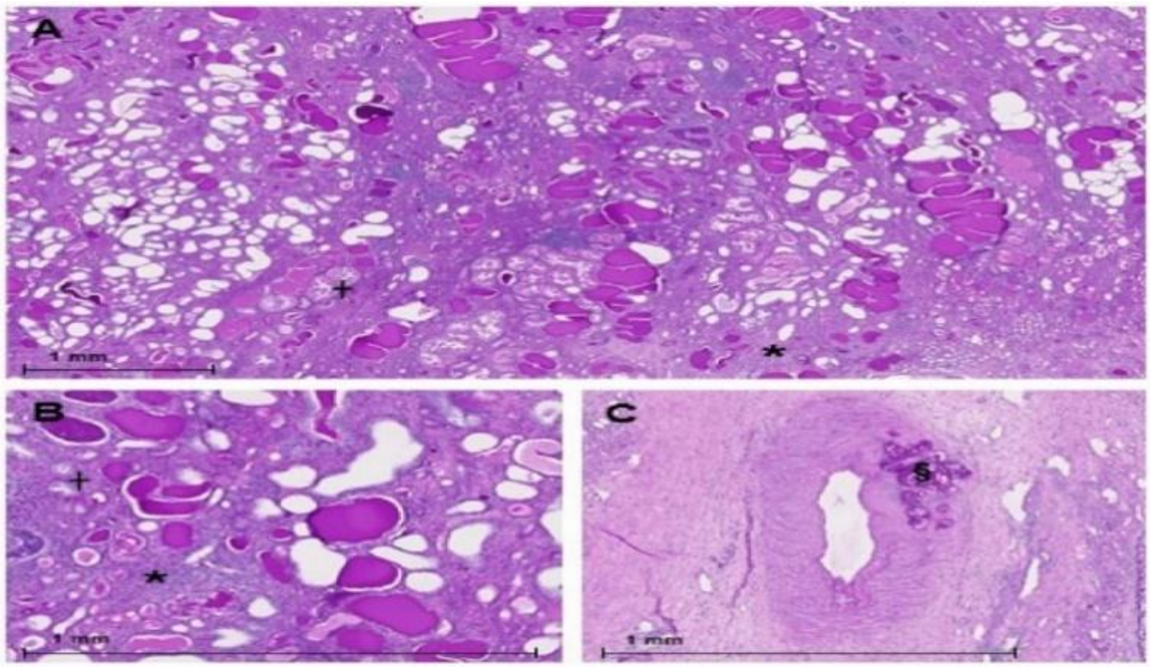
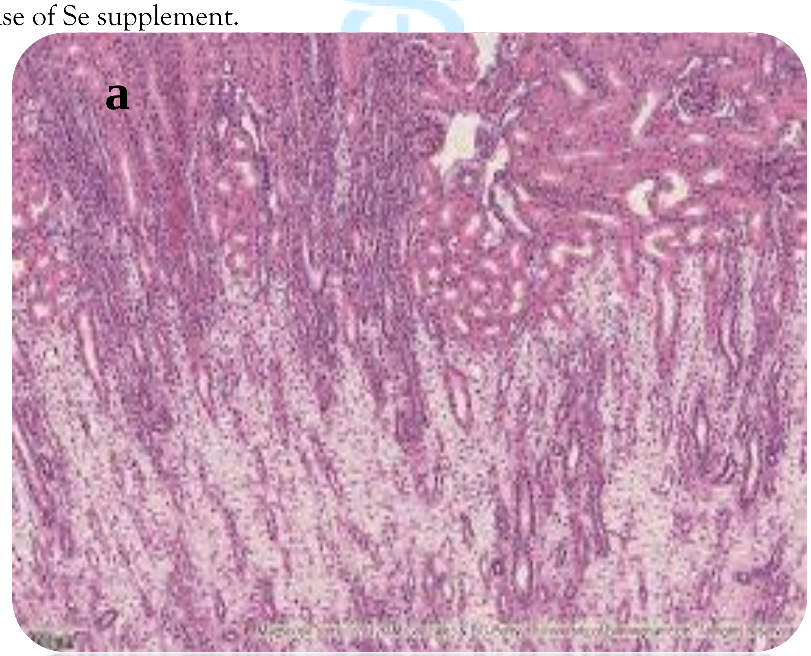


Fig 13 The right kidneys of the control group (A) and HC group (B) given no a Se supplement, showed observable regions of tubular atrophy and interstitial fibrosis. The kidney of group-C goats received supplemental Se has a noteworthy protective effect. Although there is a blockage of polyvinyl alcohol particles in the interlobar artery, but thart might be the cause of Se supplement.



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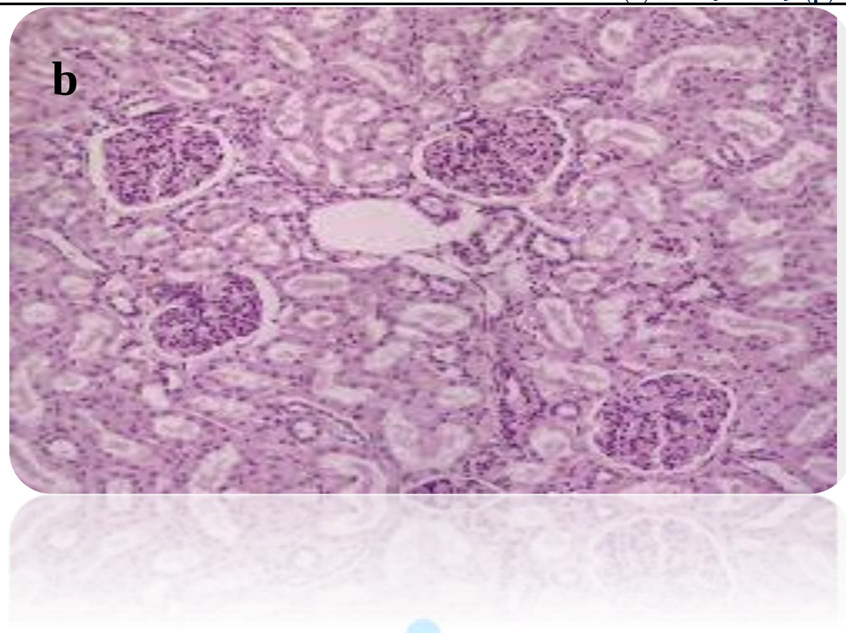

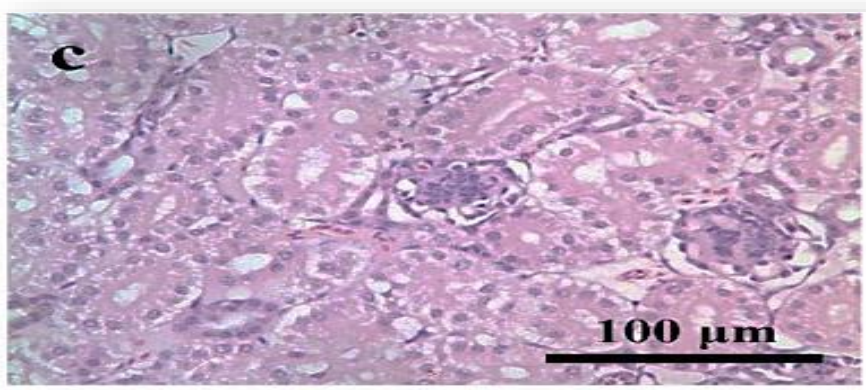

b

Fig 14 Histomorphological examination of the left kidney medulla in goats fed low concentrate (A), High concentrate (B) and High concentrate + Se-rich diet. In low concentrate diet and absence of Se (A), the kidney tissue may show slight negative effects due to oxidative stress. The kidney of goat (B) reared under a high-concentrate diet without Se supplementation showed some signs of stress and damage. However, the kidney of goats fed a Se-rich, high concentrate diet (C) revealed remarkable protective and restorative effects on renal structures.

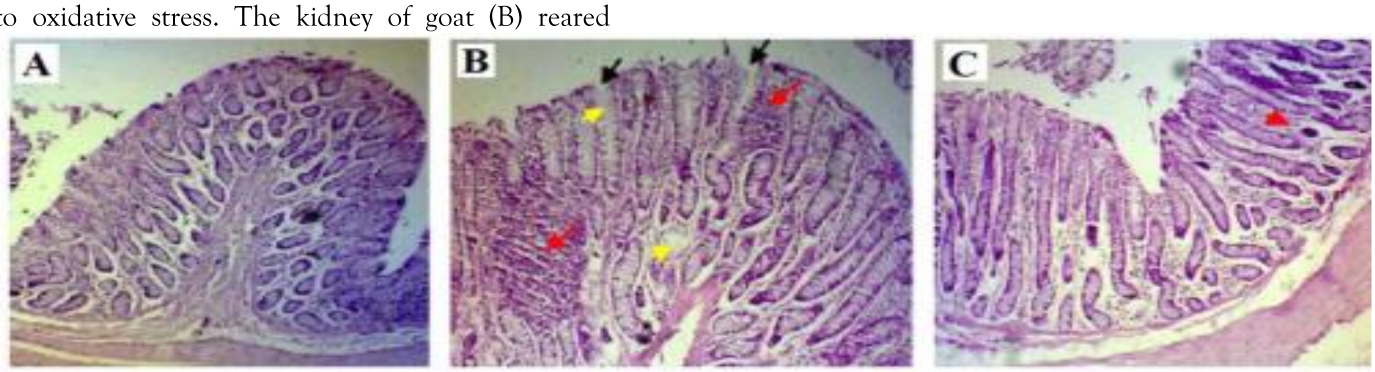

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Fig 15 Compared colonic epithelial histological damage of goats given LC, HC, and HC+Se diets. Six colonoscopy sections from each group were processed for histological assessment, and 10X magnification photos were captured. The epithelial structure in group-A (LC) appears to be rather normal (scale bar 100µm). With a scale bar of 100µm, Group-B (HC) exhibits significant inflammatory cell infiltration, goblet cell depletion, and epithelial injury as well as other histological abnormalities. Comparing Group-C (HC+Se) to the HC group, there is a noticeable decrease in histological damages, as seen by less epithelial injury, more intact goblet cells, and less infiltration of inflammatory cells (scale bar 100µm).

3.10. Se excretion per day

The study focusses on the significant effect of Se supplementation on renal function by analysing the Se excretion rates of goats fed diets containing low concentration (LC), high concentration (HC), and high concentration with Se (HC+Se). Low availability and absorption of Se was seen in the LC diet, as evidenced by the Se excretion rate of 13.88 µg/day. However, Se excretion to 26.19 µg/day (P=0.0000) increased significantly with the HC diet, indicating that increased energy consumption improves the body's ability to mobilise and eliminate Se. But Se excretion increased dramatically with the HC+Se diet, rising to 72.66 µg/day.

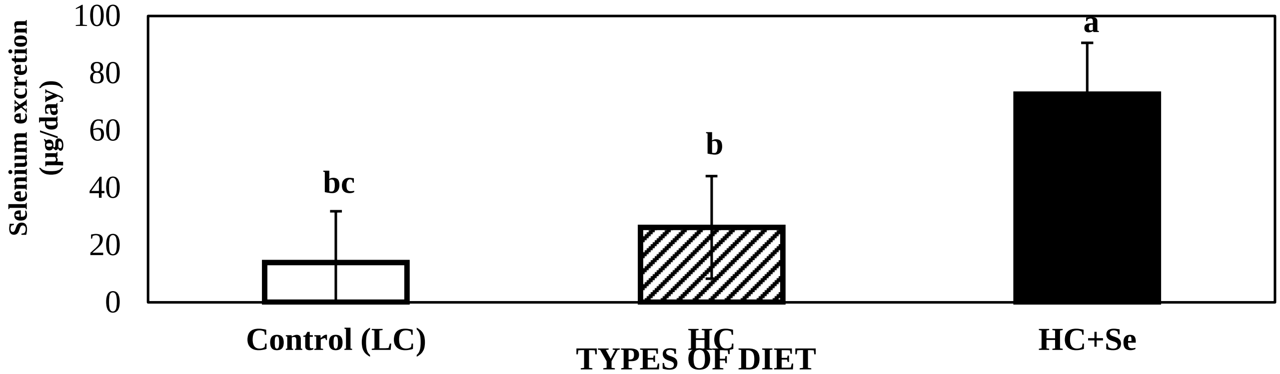

Fig 16. **Se excretion** (µg/day) in urine of goats fed on various feeding regimes such as: LC (Group A: n=6), HC (Group B: n=6) and HC+Se (Group C: n=6) was determined. The order of these diets indicates how important it is to supplement with Se in order to maximise renal function.

3.11. Blood urea nitrogen (BUN) mg/dL

The impact of meals high in concentrate (HC), high concentration (LC), and high concentration supplemented with Se (HC+Se) on goat BUN levels indicates a major role for diet in renal function and protein metabolism. The BUN was lowest given LC diet, with a mean value of 15.21 mg/dL. This may be cause reduced formation of nitrogenous waste and lower protein consumption diet. As compared to the LC group, the BUN levels in the HC group rose to 18.73 mg/dL, a statistically significant increase (P=0.0269). Out of the three groups, the HC+Se diet had the highest BUN content, rising to 22.34 mg/dL.

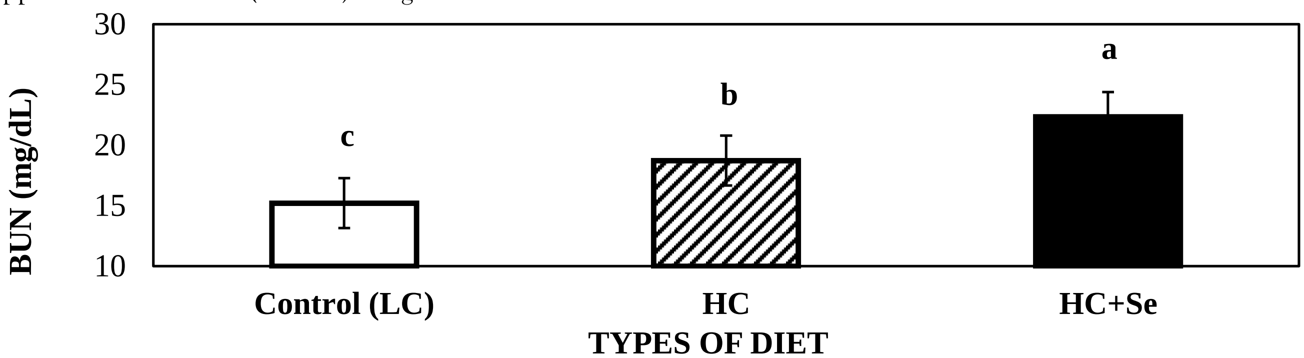

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Fig 17 Blood Urea Nitrogen (mg/dL) of goats fed on various feeding regimes such as: LC (Group A: n=6), HC (Group B: n=6) and HC+Se (Group C: n=6) was examined. The significant increase in BUN raises the possibility that adding Se to the HC diet might enhance protein metabolism, which would boost urea generation and improve nitrogen utilisation.

4.12. Glomerular Filtration Rate (GFR, mL/min)

The positive benefits of Se on kidney function are supported by the significant differences in the glomerular filtration rates (GFRs) of goats fed LC, HC, and HC+Se diets. Goats fed the control (LC) diet had the highest GFR, measuring 126.11 mL/min; goats fed the high concentrate (HC) diet had a substantially lower GFR, measuring 114.92 mL/min. The GFR did, however, decrease even further to 101.72 mL/min upon Se addition (HC+Se), indicating the part dietary Se plays in regulating renal function. fed the statistical significance (P=0.0032) of the findings, a high concentration diet may have adverse consequences that Se may be able to mitigate, but with lower GFR than goats fed LC.

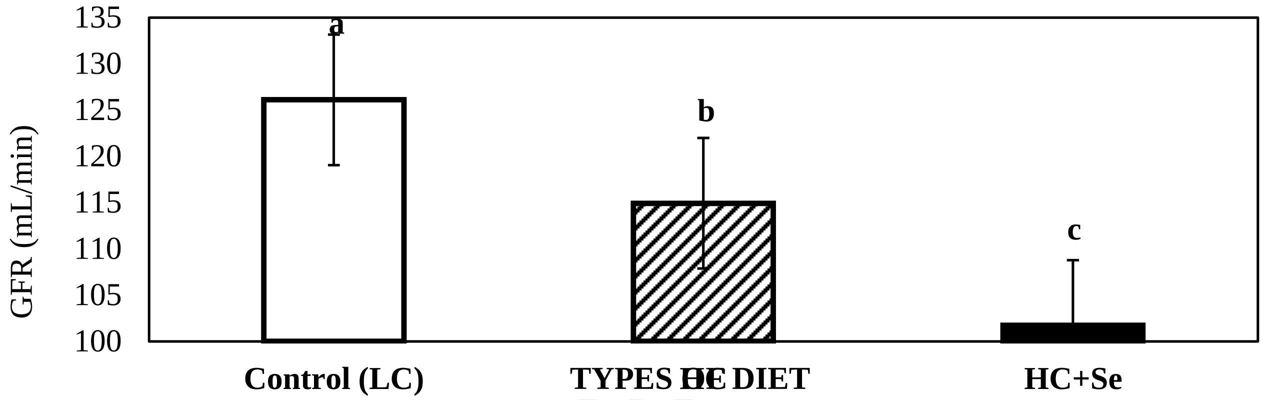

Fig 18 **Glomerular Filtration Rate (GFR, mL/min)** of goats fed on various feeding regimes such as: LC (Group A: n=6), HC (Group B: n=6) and HC+Se (Group C: n=6) was examined. While diets high in concentration may have a deleterious effect on renal function, adding Se to a diet may have some protective benefits. Nevertheless, Se still causes a reduced GFR when compared to control, suggesting that balanced concentrate and Se supplementation are necessary for goat kidney function.

DISCUSSION

The type and quality of diet have a crucial impact on morphometric and histopathological traits of goat; as high concentrate (HC) diets in addition to Se can significantly affect several bodily functions. Se affects organ growth and preservation, as shown by kidney structural changes in goats given HC+Se. In the HC+Se group, kidney weight, length, width, and thickness were significantly higher than in the HC and LC groups, demonstrating that Se is important for renal development during high-concentrate feeding (Wang et al., 2021). This protection is crucial for animals fed high-concentrate diets, which create more ROS due to greater carbohydrate fermentation and metabolic activity (Zhang et al., 2019). In goats fed HC+Se had greater kidney weight (40.32 g), length (6.48 cm), and thickness (2.76 cm) demonstrate that Sesupplementation improves tissue repair and cell proliferation. Se capacity to increase tissue integrity and minimise lipid peroxidation in cattle, especially in metabolically active organs like the kidneys, supports these findings (Lopes et al., 2020). Se is crucial to kidney health (Yuan et al., 2022). HC diets caused metabolic stress, which damaged kidney cells without Se (Jiang et al., 2019) Se interactions with selenoproteins and enzymes boost oxidative stability, which may explain HC+Se group better kidney morphology (Xie et al., 2021). It seems that Se supplementation is necessary to optimise organ development and reduce the negative effects of HC diets on kidney shape.

The renal corpuscle density, tubule cross-sectional area, and Bowman's gap width change in goat

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kidneys fed different diets. Se supplementation in HC diet demonstrates its benefits, as the HC+Se group (C) showed greater improvements than HC (B) and LC (A). Group C (11.76) renal corpuscle density was substantially higher than groups A (9.12) and B (9.69), demonstrating that Se supplementation preserves renal structure. Se antioxidant properties reduce oxidative stress, a common issue in high-concentrate diet-fed animals (Surai, 2018). As seen in groups A and B, a high-carbohydrate diet may increase oxidative stress and reduce renal corpuscle density. Se may help preserve renal corpuscles by increasing antioxidant enzymes (Wang et al., 2021). The HC+Se group showed a considerably greater glomerulus cross-sectional area (CSA) (1086.6 µm) compared to groups A (898 µm) and B (925 µm), indicating improved glomerular function and filtration capacity Se capacity to reduce kidney oxidative damage and inflammation may have improved glomeruli structure (Surai, 2018). The reduced CSA in group A (LC diet) may be due to insufficient nutritional and antioxidant intake, since Sedeficiency has been linked to poor renal function and oxidative damage (Lopes et al., 2020).

Group C showed the lowest Bowman's space diameter (92.37 µm) compared to groups A (112.65 µm) and B (107.02 µm). Group C reduced Bowman's space predicts better glomerular health since larger Bowman's spaces are associated with atrophy and dysfunction (Jiang et al., 2019). Se reduces oxidative stress and promotes cell repair, protecting the glomerulus from atrophy (Boccia et al., 2020). Group C also exhibited higher collecting, distal, and proximal convoluted tubule cross-sectional areas (CSA). Group C exhibited a considerably higher PCT (84.74 µm) than groups A (71.21 µm) and B (75.48 µm), indicating better cellular activity and nutritional reabsorption in goat kidneys after Se administration. Higher CSA suggests improved tube architecture and function retention, perhaps due to Se protection against oxidative damage from a high-concentrate diet (Xie et al., 2021). Se may prevent tubular atrophy and promote tubular regeneration, particularly in group C, by increasing tubular diameters.

The significant increase in CSA of thin tubules (27.44 µm in group C vs 19.46 µm in group A) supports the idea that Se improves renal health by reducing oxidative damage and promoting tubular structural integrity (Zhang et al., 2019). Se supplementation (group C) reduced moderate to severe tubular damage from high-concentrate meals alone (group B), showing its role in kidney shape under dietary stress. Overall, histology shows that Se addition in HC diets reduces oxidative stress, increases cell growth, and protects the kidneys from tubular and glomerular damage. This supports growing evidence that Se protects many tissues, particularly metabolically active organs like the kidney that are sensitive to food-induced oxidative stress (Wang et al., 2021).

Histopathological study of goat kidneys showed that HC+Se protected against diets lacking Se in both the HC and LC groups. The shows that diets vary in inflammation and tissue integrity and suggested that Se shortage causes kidney injury, particularly in high-concentration diets that stress renal structures. The lack of Se in LC diet caused mild but obvious histological alterations. These alterations were mostly caused by oxidative stress. The phrase "oxidative stress" refers to an imbalance between the body capacity to neutralise free radicals and produce them (Wang et al., 2021). Group A goats were more sensitive to reactive oxygen species (ROS) buildup and renal tissue oxidative injury due to the lack of Se (Surai, 2018). The damage in the goats given LC was less severe than that in the goats fed HC diet without Se (group B), but this still shows that Se protects kidney cells from oxidative damage, even when metabolic stress is low.

In goats, Se supplementation under nutritional stress strengthens renal and other organs. It also protects colonic epithelial cells, reduces inflammation, and preserves goblet cells in the HC+Se group (Gholami et al., 2021). These studies suggest animal health benefits from Sesupplementation. These data imply that high-Se diets may help kidney tissue regenerate and repair in addition to minimising tissue damage. The decrease in tubular atrophy and fibrosis in group C suggests that Semay cure diet-induced kidney damage (Lopes et al., 2020). The histopathological data show that while both low- and high-carbohydrate diets may cause kidney damage, supplementing with Sein a high-carbohydrate diet has many benefits for both restorative and preventative purposes. These results suggest the

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inclusion of Sein cow nutrition to protect renal health by highlighting the role Seplays in moderating the negative effects of high-concentrate diets on kidney shape and function. The histologically detected renal stress may be a result of this imbalance leading to the buildup of damaged cells. According to Ali et al. (2023), the HC+Se diet seems to control apoptotic pathways, which are vital for the repair and preservation of renal tissue, in addition to improving the antioxidant defence system.

Conclusions

- Increases in kidney mass, circumference, breadth, and thickness in the HC+Se group demonstrate that selenium enhances kidney morphology in goats when contrasted with HC and LC diets. Particularly, the kidneys of animals fed the HC+Se diet were larger across the board and heavier (40.32 g), suggesting improved structural integrity and, likely, function. The selenium improved kidney health in goats by decreasing oxidative stress and increasing kidney development and function.
- The kidney histology of goats demonstrates that the high-concentrate diet enhanced with selenium (HC+Se) improves renal histology more than the HC and LC diets. Importantly, the HC+Se diet increased glomerulus, proximal and distal convoluted tubule, and renal corpuscle density cross-sectional areas. Reduced histological damage and enhanced renal architecture and function result from selenium supplementation. Histological metrics demonstrate that goats' renal resilience and health improve with the HC+Se diet.

- Goat kidney histopathology studies reveal that the high-concentrate diet supplemented with selenium (HC+Se) is more protective than the HC and LC diets without selenium. The HC+Se diet dramatically decreased inflammatory responses, tubular atrophy, and interstitial fibrosis in groups A and B kidneys. Group C had less epithelial damage, undamaged goblet cells, and less inflammatory infiltration, showing that selenium may restore kidney integrity. This work supports the idea that selenium protects goat kidneys from oxidative stress and inflammation and improves renal histology.

Recommendations

- Goat farmers should supplement their goats high-concentrate diets with selenium. Selenium increases kidney size, weight, histology, antioxidant status, and haematological markers, indicating its importance.
- Regular histology and biomarker tests (such as blood urea nitrogen and creatinine levels) should assess goat kidney health. This can help diagnose kidney issues early and ensure nutritional therapy effectiveness.
- Nutritionists and feed formulators must supply balanced meals for special needs of animal. Selenium and antioxidant-rich foods should be fed to goats. This strategy may improve animal health and production.
- To understand how selenium affects kidney design and function, researchers must go deeper. Studying Se interactions with other minerals and optimal quantities might help goat producers make better food choices.

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