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Clinical and Laboratory Investigations Associated with Sheep's Allotrophagia

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Abstract

Pica is a fairly common condition in small ruminants. This study aimed to investigate changes occur in hematology, serum biochemical, mineral, MDA and thyroid hormones in pica affected sheep. The present study was conducted on 7 native breed sheep aged "1year-2.5years", of both sexes (5 females and 2 males) and five apparently healthy sheep from same locality were used as control. Clinical, physical, skin scraping and fecal samples were examined. Blood samples were withdrawn for hematologic investigation; TP, albumin, BUN, creatinine, cholesterol, and triglyceride, Zn, Cu, Ca, Ph, Mg, Malondialdehyde (MDA), T_3 and T_4 were estimated in serum and heparinized plasma. Significant reduction in RBCs, Hb, PCV, TP, and albumin, Zn, Cu, Ph and Fe along with non-significant increase in BUN, Cholesterol and MDA were recorded. Non-significant reduction in thyroid hormones observed. In conclusion, Allotrophagia appeared to be strongly correlated with mineral status in animal and have an impact on oxidative stress marker (MDA) and hemato-biochemical constituents with minimal effect on thyroid hormones.

Keywords: Sheep, Allotrophagia, Mineral, MDA, Thyroid hormones.

Introduction

Allotrophagia, also known as pica, is a fairly common condition in which animal digests material other than feeding substances as dirt, wool, wood (Youde and Huaitao, 2001). The condition is relatively known in sheep, however lambs appear to be more frequently affected (Ott et al., 1964).

Pica can be defined according to material involved, for example, coprophagia means eating fecal matter (Radostitis et al., 2007). Wool plucking behavior may be directed toward animal itself or animal trying to pluck wool of other flock's mate (Reinhardt, 2005).

Though not well-understood, many etiologies have been proposed as cause of pica, protein and mineral deficiencies are most commonly implicated (Firyal, 2007). Numerous minerals deficiencies have been postulated as causes of pica in sheep, of which zinc, copper, iron, calcium, phosphorus and manganese have gained wide acceptance (Youde and Huaitao, 2001;Meyer and Lohse,2002). A deficiency of single mineral entity is scarcely encountered under field condition, a multi-deficiencies of several minerals are more prone to occur (Hidiroglu, 1980).

A strong correlation between phosphorus deficiency and pica especially in ruminant animals has been observed (Radostitis et al., 2007), however other reports did not detect this relation (Akgul et al., 2000). Peroxidation of lipid is thought to play an integral role in developing anemia (Saleh, 2008). Polyunsaturated fatty acids are susceptible to oxidation process which consequently leads to lipid peroxidation and Malondialdehyde (MDA) production (Crongaj et al., 2010). Free radicals resulting from peroxidation are usually eliminated from body by a group of enzymes component of which involve many important trace elements such as zinc and copper (Grotto et al., 2009).

Thyroid hormones have direct impact on growth and metabolism (Guton, 1986). The decrease in thyroid hormones activities are found to cause anemia, alopecia and emaciation in lambs (Sokkar et al., 2000).

Clinically, pica can cause unthriftness, poor condition, wool loss, loose feces and limb abnormalities (Akgul et al., 2000). Pica can have a serious consequences on animal health status, pica can pinpoint an error in diet and serious mineral imbalance which consequently will affect future production and reproduction of animals (Chiezey, 2010).

So this study was performed to investigate changes occur in hematology, serum biochemical constituents, mineral, MDA and thyroid hormones in pica affected sheep.

Material and Methods

1.1. Animals

The present study was conducted on 7 native breed sheep aged between 1year-2.5years, of

both sexes (5 females and 2 males). The sheep were owned privately in Giza Governorate, Egypt and the main complaint was unthriftness, poor condition and tendency to eat wool though deworming treatment was applied. Five apparently healthy sheep from same locality were used as control. Fecal samples from both healthy and affected sheep were examined via direct microscope examination and sedimentation and floatation technique to exclude internal parasitism (Soulsby, 1983).

1.2. Skin examination

Thorough examination of skin was applied for exclusion of external parasites, skin scraping was performed to exclude mange and dermatophytosis (Radostits et al., 2000).

1.3. Hematologic evaluation

Blood from jugular vein was collected on EDTA-containing tube for hematologic evaluation, blood smears stained with Giemsa was evaluated to exclude blood parasites (Feldman et al., 2000).

1.4. Heparinized plasma

Heparinized plasma samples were used for estimation of Malondialdehyde "MDA" using specific test kits (Bio-diagnostic, Egypt). Briefly, Thiobarbituric acid reacts with MDA in acidic condition for 30 minutes at 95ºC to form reactive product with pink coloration can be measured spectrophotometrically.

1.5. Serum samples

Blood from jugular vein was collected on plain tube and kept in slanted position then centrifuged to separate a clear serum samples, samples were divided into 3 portions (for mineral, biochemical constituents and thyroid hormones).

1.5.1. Mineral profile evaluation

Serum samples were used to estimated copper, zinc, iron, calcium, phosphorus and magnesium using specified test kits (Spectrum diagnostic-Egypt). Evaluation was performed using spectrophotometer according to manufacturer's instructions.

1.5.2. Selected biochemical constituents

Estimation of total protein, albumin, BUN, creatinine, cholesterol and triglycerides was performed on serum samples spectrophotometrically using specific test kits according to instructions provided by manufacturer ((Spectrum diagnostic-Egypt).

1.5.3. Thyroid hormones estimation

Serum samples for evaluation activity of T_4 and T_3 concentration using enzyme immune assay test kit (BIOS, Chemux BioScience, INC. San Francisco, CA, USA) EIA test according to manufacture instructions**.**

1.6. Statistical analysis

Unpaired T-test (STATISTICA for Windows, version 5.1., StatSoft, Inc.) was used, $P \leq 0.05$ considered significant.

Results

Clinical presentations of affected sheep showed wool plucking, areas of alopecia, poor condition, pale mucous membrane and varying degrees of dermatitis and one case showed limb abnormality (Figure 1). Three animals showed signs of loose feces. Parasitological examination, skin scraping and blood smears revealed negative findings.

Results of hemato-biochemical evaluation are shown in table 1. Significant reduction in PCV, Hb and RBCs were observed in affected sheep compared to healthy sheep. Concerning serum biochemical constituent, significant reduction in total protein and albumin with non-significant increase in BUN and cholesterol were recorded.

Results of mineral profile and MDA are shown in table 2. Concerning mineral profile significant decrease in Cu, Zn, Fe and Ph were observed in affected sheep compared to healthy ones. Non-significant increase in MDA was observed in affected sheep compared to control sheep.

Concerning thyroid hormones activities (table 3), non-significant decrease in T3 and T4 were observed, however the decrease considered to be not statistically significant.

Figure 1. A) Area of wool loses and poor condition in pica affected sheep. **B)** Area of alopecia with degree of dermatitis **C)** Wool eating behavior and poor condition in pica- affected sheep. **D)** A case showing limb abnormality and wool eating behavior.

Table 1. Hematologic and serum biochemical findings in control and affected sheep

Parameter	Control sheep (n=5)	Affected sheep (n=7)
RBCs	10.09± 0.496	7.56±0.371*
Hb	10.532±0.561	8.35±0.48*
PCV	32.68±2.04	27.59±0.80
WBCs	9.510±0.69	9.98±0.57
Neutrophil	25.20±5.58	29.43±4.83
lymphocytes	53.60±5.14	51±4.11
MCV	31.085±1.57	28.59±1.41
MCHC	34.19±2.54	32.70±1.89
TP	6.89±0.357	6.06±0.124*
Albumin	3.40±0.163	2.77±0.134*
BUN	18.140±1.967	19.40±1.6
Creatinine	1.16±0.081	1.13±0.078
Cholesterol	62.52±3.13	67.8±3.89
Triglycerides	19.05±1.09	21.47±1.24

*P≤0.05

Table 2. Minerals and MDA status in control and affected sheep

Parameter	Control sheep (n=5)	Affected sheep (n=7)
Copper	91.60±12.33	57.09±8.09*
Zinc	101.8±11.63	66.89±4.65*
Iron	104.84±2.32	84.100±2.94*
Calcium	10.9±0.288	10.86±0.228
Phosphorus	6.38±0.402	4.85±0.35*
Magnesium	2.33±0.233	2.09±0.36
MDA	3.32±0.28	4.22±0.35

*P≤0.05

Table 3. Thyroid hormones profile in control and affected sheep

Parameter	Control sheep (n=5)	Affected sheep (n=7)
T3 ng/ml	3.57±0.19	3.32±0.21
T4 ug/dl	12.58±2.33	11.75±1.33

Discussion

Pica is a worldwide disorder and not restricted to certain geographical location, though many causes have been proposed, mineral deficiencies remain most acceptable justification (Garg et al., 2013). Wool eating is a form of allotrophagia occur in ovine particularly of young age (Icen et al., 2008).

In this study, clinical presentation was wool plucking, poor condition, varying degrees of dermatitis and alopecia, pallor mucous membranes also observed, similar presentations were recorded in other literatures (Youda, 2002, Icen et al., 2008). Deficiency of zinc was correlated to wool eating problem and alopecia in sheep (Nelson et al., 1984). Pale mucous membrane could be associated with anemia (Radostitis et al., 2007). Poor growth is a result of reduction in lysyl oxidase and cytochrome C, moreover, changes in wool

is primary linked to Cu-Zn dismutase which play a role in melanin formation (Rucker, 2007). Reduction in zinc level associated with decrease animal efficacy to utilize food, impair growth rate and causing dermatitis which manifested as parakeratosis via histopathology (Harvey, 2008).

Upon fecal and skin scraping examination, investigation failed to establish parasitic involvement, this findings conceded with other reports that suggest pica is not correlated with parasitism (Abd El-Raof and Ghanem, 2006).

Reduction in RBCs, PCV and hemoglobin content were recorded, there was controversial reports regarding erythrogram in sheep's allotrophagia, though some authors found significant reduction in PCV and Hb only (Ebrahim, 2015), others detected no change in erythrogram (Nelson et al., 1984), however, reduction in erythrogram was recorded in other reports (Abd El-Raof and Ghanem, 2006). The disturbance in iron metabolism consequent to copper deficiency may explain reduction in erythrogram (Church and pond, 1988). Deficiency in copper with consequent reduction in copper-containing cytochrome oxidase inside mitochondria that slow down reduction of iron and subsequently reduce "heme" synthesis that utilize iron (Harvey, 2008). Furthermore, the decrease in Cu-Zn SOD associated with reduction in "heme" synthesis (O'Dell and Sunde, 1997).

Anemia was constant finding in sheep with zinc deficiency (Al-Saad et al., 2010). Results of red cell indices are suggestive of normocytic normochromic type, in sheep, zinc deficiency is known to cause anemia of normocytic normochromic nature (Al-Saad et al., 2010, Ebrahim, 2015). Anemia associated with copper deficiency is usually hypochromic microcytic, though normocytic anemia was also reported, moreover normochromic macrocytic anemia was recorded in both bovine and ovine (Brewer, 1987).Though no changes were recorded regarding leucogram in pica (Arlington et al., 1996).

Concerning protein profile, a reduction in total protein and albumin were recorded, inappetence as a result of copper deficiency may be implicated, and copper is thought to elevate intravascular permeability with consequent albumin decrease (Abd El-Raof and Ghanem, 2006). Non-significant increase in BUN was observed, it has been concluded that BUN increases with cases of copper deficiencies, however, the exact mechanism is not well understood (Randhawa and Brar, 1998). Moreover, BUN has tendency to elevate in sheep with low body condition score (Caldeira et al., 2007). Non-significant increase in cholesterol level was observed in this study, Cu deficiency is known to cause alteration in lipid content, particularly cholesterol, and it was proven that Cu deficiency is associated with hypercholesterolemia due to elevation in HDL and LDL (Engle et al., 2000). Zinc causes a decrease in utilization of glucose and subsequently lipid metabolism alteration manifested by increase cholesterol (Fekete and Brown, 2007).

Regarding mineral profile, significant reduction in copper, zinc, phosphorus and iron were recorded, similar results were found (Fahmy et al., 1980, Abd El-Raof and Ghanem, 2006, Ebrahim, 2015), however, Akgul et al., (2001) observed non-significant changes in iron. Copper is an important element for wool (McDowell, 1999), in general, mineral can affect wool especially copper and zinc, they exert their effect by disruption of food uptake and rumen function or straight effect on sheep's metabolism (Freer and Dove, 2002). Reduction of copper level leads to iron metabolism impairment, it was correlated with decrease release of iron from enterocytes to plasma due to decrease hephaestin which facilitate exportation of iron via ferroportin (Wessling-Resnick, 2006).

Moreover, copper affects mobilization and utilization of iron, copper plays a part of redox cofactor which associated with ferroxidases (Harvey, 2008). A decrease in phosphorus level was observed, phosphorus is thought to be the number one cause of pica (Radostitis et al., 2007). Zinc deficiency and its concurrency with Ph deficiency has been recorded (Ellis et al., 2016). Non-significant increase in MDA was observed, copper is known to play a role in defense against oxidative injuries via Cu-Zn SOD activities (Wu and Meininger, 2002). Furthermore, MDA is recorded to elevate in Pica affected cattle (Elshahawy and Aly, 2016). MDA is byproduct of lipo-peroxidation

and characterized by its stable nature (Moore et al., 1995). When levels of peroxidation process surpass antioxidant system, an assault on cell membrane undertake, that's why MDA estimation is widely sought after in oxidative process pathology (Grotto et al., 2009). MDA level was recorded to increase in association with copper deficiency (Duan et al., 2010).

Non-significant decrease in T3 and T4 were recorded, these findings conceded with fact that deficiency in zinc does not appear to affect thyroid hormones metabolism (Harvey, 2008). Thyroid hormones found to be altered in association with other deficiencies as selenium (Dalir-Naghadeh and Rezaei, 2008). Moreover, in rat's model, Cu deficiency was found to have minimal impact on thyroid hormones (Kralik et al., 1996).

2. Conclusion

Allotrophagia in sheep appeared to be strongly correlated with mineral status and have an impact on oxidative stress marker (MDA) and hemato-biochemical constituents with minimal effect on thyroid hormones.

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الملخص العربى

الاستقصائات السريرية والمخبرية المرتبطة بالتروفاجيا فى الغنم

نهى يوسف سالم

مدرس الامراض الباطنه,, قسم الامراض الباطنه والمعديه,, كليه الطب البيطري جامعه القاهرة

اجريت هذه الدراسه على عدد سبعه خراف مصابه باعراض نقص الشهيه و اكل اغراض غير مخصصه للاكل مثل الصوف و خمسه خراف سليمه اكلينيكيا. تم دراسه التغيرات فى الدم والبيوكيمسترى والتغييرات فى المعادن وهرمونات الغده الدرقيه. تم ملاحظه تغيرات الدم عباره عن انخفاض نسب الهيموجلوبين وكرات الدم الحمراء وانخفاض معنولى فى البروتين والابيومين والنحاس والزنك والحديد والفوسفور وتغيير غير معنوى فى هرمونات الغده الدرقيه و الملودنديالدهيد والكوليستيرول. التروفاجيا مرتبطه بتغيرات فى حاله المعادن فى الحيوان ولها تاثير على الملودنديالدهيد وتغيرات الدم و البيوكيمسترى ولها تاثير غير ملحوظ وضعيف على هرمونات الغده الدرقيه