Effect of Oral Administration of Potassium Iodide on Clinical Status and Metabolic Profile in Sheep

SABRY A. MOUSA1*, ENAS ELMELIGY2, DALIA HASSAN3, AHMED HAFEZ4, FATMA ALI5, ARAFAT KHALPHALLAH6

1Department of Medicine and Infectious disease, Faculty of Veterinary Medicine, Cairo University, P.O. Box 12211, Giza, Egypt; 2Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt; 3Department of Animal & Poultry Hygiene and Environmental Sanitation, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt; 4Department of Pharmacology, Faculty of Veterinary Medicine, Assiut University, Asswan 81528, Egypt; 5Physiology Department, Faculty of Veterinary Medicine, Assiut University, Asswan 81528, Egypt; 6Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt.

Abstract | The present article discussed the relationships between thyroidal hormonal changes, rumen functions and metabolism, serum biochemicals assays, electrolytes and blood pictures in sheep in Egypt either before or after potassium iodide (KI) adding to their rations through long term study extended for successive 65 days. The study was conducted on fattening Osimi sheep (n=19) with ages ranging between 6-11 months. Animals were supplied with KI salts dissolved in distilled water in the morning with a dose of 0.15 mg/kg in addition to 0.093 mg iodine /kg DM/day for successive 65 days. The examined sheep were undergoing thorough investigations included clinically, laboratory, rumen functions and body gain estimation according to the following schedule; zero, 15th, 30th and 65th days. The study reported significant changes in rumen metabolic functions through the remarkable improvement in each of the protozoal ciliate density and TVFAs, and reduced ruminal ammonia as well as maintaining normal pH. Most of blood picture indices showed clear improvement particularly red blood corpuscles and haemoglobin concentration. Thyroid functions were clearly affected with long-term 65 days KI supplementation as thyroid hormones were significantly reduced in their concentrations and reached their lowest values at day 65, however, they were still not lower than their reference values. Serum biochemicals showed no changes except for glucose that showed significant improvement. Long-term KI supplementation had a great impact on serum blood urea nitrogen and alkaline phosphatase through their significant serum concentrations reduction. A significant improvement in body weight gain was reported in treated sheep during the current study with a reported highest body weight gain at the 65th day. In Conclusion, long-term 65 days KI supplementation induced variable significant changes in sheep mainly rumen metabolic functions, blood picture indices, thyroid hormones functions and body weight gain.

Keywords | Sheep, Thyroid functions, Rumen metabolism, Total protozoal count, Total volatile fatty acids.

INTRODUCTION

Iodine in an inorganic form (KI, KIO3, CaIO3) was one of the most commonly used in mineral supplements for farm animals. Furthermore, Greater concern with its organic compounds, e.g. ethylenediamine dihydroiodide (EDDI), iodine on an oil base–iodinated fatty acid esters (Herzig et al., 2000) was occurred. The advantages of these organic compounds of iodine were of stable biological activity in animals.

As a mineral element, iodine was not synthetized within the body. Soil and consequently plants were the first sources of iodine. If the iodine concentrations of soils were rela-
Iodine (I) intake from the environment was that the critical factor, which affected the function of the thyroid (Peksa et al., 2013; Ong et al., 2014; Medrano et al., 2016), milk production, growth intensity, wool growth and animals reproductivity (Pugh and Baird, 2012). In accordance with NRC (2001) the recommended iodine content during a feed ration for sheep and cows was merely 0.5 mg/kg.

The excessive iodine amounts in farm animals may cause iodism which was characterized by loss of appetite, lacrimation, respiratory and reproduction problems, hyperthermia, hypoglycaemia, and a drop in milk yield (Huszenica et al., 2002), decrease in feed intake resulting in a subsequent drop by weight gains (Herzig and Suchý, 1996), lower concentration of haemoglobin (Hb) in blood and iron in liver (Kirschmann and Kirschmann, 1996).

Production of thyroid hormones was that the most vital function of the thyroid. They included triiodothyronine (T3), thyroxine (T4), and thyroid stimulating hormone. Thyroid hormones greatly affected the entire metabolism of organism. the main function of the thyroid organ was to secrete TH, which facilitated the body utilize energy, keep warm and maintained the vitals (brain, heart, muscles, and other organs) to figure efficiently (Nabeel et al., 2017).

The normal synthesis of thyroid hormones containing iodine depended on the supply of adequate quantities of exogenous iodine. Due to its many pharmacological actions, iodine participates were not only an important substrate for hormone biosynthesis. Therefore, the effect of iodine on the thyroid might be a combination of the consequences of both pharmacological action and iodine as a substrate for hormone (Peksa et al., 2013).

The microbial populations including ciliate protozoa within the rumen consisted mainly of bacteria, protozoa and fungi that involved within the digestion of feed the rumen (Akbar et al., 2009).

Estimating blood biochemical parameters, hormone concentrations, and enzymes activities of animals clearly described the nutritional and health status, before the mentioned problems were even visible within the animal (Antunović et al., 2009).

Several studies concerned with the changes in thyroid hormones in sheep as a result to supplementation with cadmium (Badiei et al., 2009a), lead (Badiei et al., 2009b), selenium (Novoselec et al., 2017) or iodine (Aumont et al., 1989; Travniecek et al., 2010).

Furthermore, several studies focused on the effect of iodine supplementation on ewe like Myers and Ross (1959); Dušová et al. (2014). Dušová et al. (2014) evaluated the influence of high nutritional intake of iodine in ewes (above the upper limit of the permitted EU standard, 2005, of 5 mg/kg of 88% of dietary DM) on some haematological and biochemical parameters of the blood of ewes and their lambs, however, the connection between iodine supplementation and changes in rumen metabolism functions parameters, thyroidal hormones, blood electrolytes and weight needed further elucidation in sheep in Egypt.

According to the previous studies, this article discussed the relationships between thyroidal hormonal changes, rumen functions and metabolism, serum biochemicals assays, electrolytes and blood pictures in sheep in Egypt either before or after iodide (KI) adding to their rations through long term study extended for 65 days.

MATERIALS AND METHODS

Animals
The study was conducted on fattening Osimi sheep (n=19), with ages ranging between 6-11 months. They were females. This study was carried out at the farm of Faculty of Agriculture, Cairo University, Egypt from May 2017-Jule 2017. The animals were clinically examined and sampled for each of ruminal fluid analyses, haematological procedures, serum biochemical assays and estimation of body weight gain at days 0 (Before KI supplementation), 15, 30 and 65. Body weight gain was estimated using ordinary balance by Kg.

Ethical Approval
All animal procedures performed during this study were conducted in accordance with Institutional Animal Care and Use Committee guidelines of Cairo University which basically conform to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health within the USA (NIH publication No. 86-23, revised 1996).

Feeding Strategy
Animals were supplemented with KI salts dissolved in distilled water in the morning with a dose of 0.15 mg/kg according to NRC (1985) in addition to 0.093 mg I/kg DM. on a daily basis for successive 65 days. Yellow corn, soya bean, sunflower, wheat bran and premix with chemical analysis were fed to these animals (Table 1). The examined sheep was fed the basal diet containing 60 % concentrate mixture and 40 % good quality hay. Basal diet was formulated according to nutrient requirements of sheep (NRC, 1985) to meet the nutrient requirements of investigated sheep.
Table 1: Chemical composition of the experimental ration and premix

| Ingredient    | Percentage % | Ingredient    | Percentage % |
|---------------|--------------|---------------|--------------|
| Fat           | 3.22         | Calcium       | 33.68        |
| Fibers        | 5.69         | Sodium        | 13.28        |
| Moisture      | 10.3         | Phosphorous   | ND           |
| Ash           | 4.4          | Potassium     | 177.8        |
| Protein       | 15.8         |               |              |

Drugs KI salt manufactured by El-Gomhoreya Pharmaceutical and Medical Supplies (Egypt) were available in jars of 0.15 mg/kg consistent with NRC (1985) additionally to 0.093 mg I/kg DM. daily (Angelow et al., 2011).

CLINICAL EXAMINATION
All sheep underwent thorough clinical examination including general inspection, pulse, temperature, rate of respiration, mucosa, lymph nodes, ruminal motility and parasitological examination consistent with Jackson and Cockcroft (2002); Nagy and Pugh (2012).

RUMEN JUICE SAMPLES AND ANALYSES
Collection of rumen samples was administered just before morning feeding (Khaled and Baraka, 2011). From each animal, 50 ml of rumen fluid was collected from each sheep on days 0, 15, 30 and 65 of experiment employing a rubber stomach tube. Rumen pH (using SMP1 pH-meter) decided immediately after the gathering of fluids; then samples were sieved, divided and stored for determination of total protozoa count consistent with the tactic described by Dehority (1984), ammonia concentration consistent with Conway (1957); Zapletal (1967) and volatile fatty acids concentration consistent with Warner (1964); Cottyne and Boucque (1968). Centrifuged strained rumen liquor supernatant was stored in deep-freeze at -20°C until assayed. Sample for estimation of TVFs and ammonia, was divided into two portions: the primary portion (2ml) for determination of ammonia nitrogen level after preservation by adding liquid paraffin, the second portion for total volatile fatty acids (TVFAs) (2ml) after preservation by adding 2ml phosphoric acid and 1ml acid N/10 according to Warner (1964).

For determination of total protozoa count, a well-mixed sample of the ruminal content was immediately fixed with an equal volume of 18.5% formalin (Dehority, 1984). For total and differential protozoal count, solution of methyl green formalin saline (MFS) was used for staining and storage of an aliquot of every sample (Ogimoto and Imai, 1981; Gürelli 2014b). MFS was used as a nuclear stain, and Lugol's iodine was won to indicate skeletal plates (Gürelli and Ito, 2014; Gürelli et al., 2016). A Neubauer haemocytometer counting chamber was used for counting ciliate densities or concentrations. This counting chamber has slender grooves cut at regular intervals. The number of cells per 1 ml of rumen contents are often calculated by subsequent formula: N = 10/4 × a × d (N: number of ciliates per 1 ml of ruminal contents, a: ciliates number in four divisions of the Neubauer hemocytometer, d: sample dilution) (Selim et al., 1996a; Gürelli, 2014b; Gürelli et al., 2016).

BLOOD SAMPLES AND LABORATORY ANALYSES
For laboratory evaluations, two blood samples were collected from jugular veins of each sheep before morning feeding; the first sample was placed in a vacutainer tube containing ethylene diamine tetra-acetic acid (EDTA) as anticoagulant for hematological analysis, and the second sample was collected in a plain tube for biochemical analysis (Coles (1986); Khaled and Baraka, 2011). Complete blood picture including red blood corpuscles (RBCs), total leucocytic count (TLC), Hb, packed cell volume (PCV), mean corpuscular values were manually estimated according to Coles (1986); Harvey (2001); Latimer et al. (2011). Mean corpuscular values included mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular Haemoglobin concentration (MCHC). After centrifugation of the second blood sample, serum samples were collected and then frozen at −20°C until thyroidal hormone levels measurements and other serum biochemical assays were conducted. T3 and T4 were estimated by solid phase time-resolved fluoroimmunoassay described by Boland (Boland et al., 2008). Serum concentrations of sodium, potassium, calcium and phosphorus, glucose, alkaline phosphatase (ALP), blood urea nitrogen (BUN), and creatinine were assessed by using the Optizen32220 UV/Visible spectrophotometer (Mecasys, Korea) according to the method described in kits and reagents were obtained from Bio diagnostic company, Egypt.

STATISTICAL ANALYSIS
The data were analyzed by using the SPSS statistical software packaged program for Windows (ver. 16.0; SPSS, USA). Paired t-test was used for analyzing the obtained data were analyzed by. The significance of differences between the means at zero day and the other day i.e. 15th, 30th or 65th days, was evaluated by Dunnett’s test. Values were expressed as means±SD at p<0.05.
Table 2: Mean values (M±SD) of body weight, temperature, pulse and respiration in investigated sheep (n=19) either pre or post treatment.

| Follow up period | Body weight (Kg) (39-40)1 or (38.5-40)2 | Temperature (C) (70-90)1,2 or (60-70)1,2 | Pulse (Beat/min) (10-20)1 or (20-30)2 | Respiration (/min) (2-4)1 | Rumen (cycle/2 mins) (2-4)1 |
|------------------|---------------------------------------|----------------------------------------|---------------------------------------|------------------------|------------------------|
| Day 0            | 30.71±0.9                             | 39.32±0.39                            | 86.68±2.98                           | 24.74±4.09            | 2.53±0.69              |
| Day 15           | 37.36±1.15                            | 39.26±0.30                            | 86±3.14                              | 24.37±4.3             | 2.53±0.61              |
| Day 30           | 42.60±1.34                            | 39.27±0.33                            | 85.1±2.77                            | 25.11±3.65            | 2.32±0.48              |
| Day 65           | 45.73±1.41                            | 39.31±0.36                            | 85.37±2.83                           | 24.53±4.26            | 2.47±0.61              |

Significant when the values at day 0 (Pretreated group) compared with the values at days 15, 30 or 65 (P<0.05). 1Reference value according to Radostits et al. (2007). 2Reference value according Jackson and Cockcroft (2002).

Table 3: Mean values (M±SD) of rumen fluid parameters in investigated sheep (n=19) either pre or post treatment.

| Follow up period | TPC (×10^4 ml) or (28.1±20.0)3 or (28.13±4.13)1 or (67 [16-200])2 | PH (6.06±0.05)1 or (6.4-6.8)4 or (6.5 to 7.5)5 | TVFs (mmol/l) (70-150)6 or (103.70±1.16)7 or (158.13 ± 9.09)8 | Ammonia (mg/L) (50-300)9 or (130±2.35)7 or (40-240)6 or (89.3-93.3)10 |
|------------------|-------------------------------------------------|-----------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| Day 0            | 31.73±2.64                                      | 6.41±0.07                                     | 45.86±5.35                                                   | 302±4.08                                                      |
| Day 15           | 66.94±5.99                                      | 6.94±0.06                                     | 63.44±3.98                                                   | 221±10.45                                                    |
| Day 30           | 36.26±4.37                                      | 6.90±0.04                                     | 62.68±2.96                                                   | 132±8.79                                                     |
| Day 65           | 13.68±2.35                                      | 7.26±0.03                                     | 75.36±4.10                                                   | 124±8.13                                                     |

TPC: total protozoal count. TVFs: total volatile fatty acids. *Significant when the values at day 0 (Pretreated group) compared with the values at days 15, 30 or 65 (P<0.05). 1Reference values according Baraka (2012). 2Reference values according to Selim et al. (1996b). 3Reference values according to Gürelli et al. (2016). 4Reference values according to Jasmin et al. (2011). 5Reference values according to Navarre et al. (2012). 6Reference values according to McDonald et al. (2011). 7Reference values according to Akbar et al. (2009). 8Reference values according to Khaled and Baraka (2011). 9Reference values according to Satter and Slyter (1974). 10Reference values according to Rianto et al. (2005).

RESULTS

CLINICAL FINDINGS

The most common clinical findings of the examines sheep during the current work showed normal appetite, normal mucous membranes (Bright red without abnormal discharge), no oral lesions and normal lymph nodes. Abnormal lung sounds, diarrhea, ataxia and signs of dehydration were not reported. The Capillary refill time (CRT) was < 2 S revealed normal viscosity of the blood with fluid and electrolytes balance.

A significant improvement in body weight gain was reported in treated sheep during the current study after treatment with KI as body weight was remarkably (p<0.05) increased at days 15, 30 and 65 comparing with their values at day 0. The highest body weight gain was reported at the 65th day (Table 2).

Temperature, pulse, respiration and rumen movement showed no remarkable changes throughout the study. These findings were within the normal physiological reference values to assure that these animals were having good health and soundness during the 65 days of the experiment (Table 2).

RUMEN FLUID METABOLISM AND FUNCTIONS

The density of rumen ciliate protozoa in investigated sheep was significantly (p<0.05) increased at day 15 post-treatment with KI when they were compared with their values at day 0 before KI treatment. This total protozoal count (TPC) was significantly (p<0.05) reduced at day 30, hence, it reached its lowest concentration at day 65 when these values were compared with the TPC values at day0. The concentrations of rumen ciliate protozoa in sheep through the study were within the normal physiological reference values except for their density at day 65 which was lower than these reference values (Table 3).

Comparing to values at day 0, rumen constituents (Table 3) showed that rumen pH was significantly elevated (p<0.05) after treatment at days 15, 30 and 65 but generally they were within the reference range of rumen pH in sheep. These changes were attributable to the addition of KI.

Rumen TVFs showed significant increase (p<0.05) in their concentration at the 15th day comparing to their concentrations at the 0 day. This remarkable (p<0.05) improvement in the mean values of TVFs continued in during the experiment at days 30 as well as it reached its highest values at day 65. TVFs were within the normal physiological range (Table 3).
Rumen ammonia levels were remarkably changed after supplementation of the examined sheep with KI as their levels were significantly (p<0.05) dropped post-treatment starting from the 15th day when they were compared with ammonia values at day 0. This significant (p<0.05) reduction in rumen ammonia concentration was more clear at days 30 and 65 comparing to their values at day 0. Rumen ammonia levels during the present work were higher than some reference values however they were within the normal ranges according to other references (Table 3).

**Whole Blood Picture Indices**

The present results reported an improvement in most of whole blood picture indices at the 15th, 30th and 65 days following treatment with KI except for MCHC and TLC that showed no remarkable changes after treatment when their values compared with those at day 0 before treatment. RBCs were significantly (p<0.05) increased at days 15, 30 and 65 when they were compared with their values at day 0. RBCs was gradually increased regarding to KI treatment and so they reached their highest values at day 65. RBCs values were lower than the reference values at days 0 and 1, hence, they were within the normal range at the 30th day [14.33±0.34] and the 65th day (Table 4).
MCV and MCH were significantly (p<0.05) decreased in response to treatment with KI particularly at days 30 and 65 comparing to their values at day 0. MCV and MCH were within the reference range at days 30 and 65 while they were higher than the normal values at days 0 and 15 (Table 4).

**SERUM BIOCHEMICAL ASSAYS**

Thyroid hormones functions showed remarkable changes as a response to adding of KI to sheep diet where T3 and T4 showed significant (p<0.05) reduction in their concentrations after treatment at days 15 and 30 when compared with their values at day 0. This clear drop (p<0.05) in serum levels of thyroid hormones comparing to day 0, continued until reached their lowest values at day 65. In contrast, serum concentrations of T3 throughout the present study were higher than the reference values either pretreatment or post-treatment, hence, serum T4 levels were within the normal reference ranges (Table 5).

Serum concentrations of electrolytes including sodium and potassium were not significantly changed where they were within the normal physiological ranges. At the same time blood levels of calcium and phosphorous also showed no remarkable changes through the current study whether before or after KI supplementation. Calcium levels showed slight increase while those of potassium showed slight decrease after treatment at days 15, 30 and 65. However, they were within the reference ranges (Table 6).

Serum levels of glucose were significantly (p<0.05) increased after treatment particularly at days 30 and 65 when compared with their levels at day 0 (Pretreatment). The obtained results reported higher levels of glucose post-treatment than the reported reference values (Table 6).

BUN and creatinine concentration were decreased at days 15, 30 and 65 when compared with their values at day 0. The reduction in serum levels of BUN was significant (p<0.05), hence, it was not significant (p>0.05) for serum creatinine. Serum levels of BUN after treatment at day 15, 30 and 65, were lower than their reference values, hence, those of creatinine were higher than their reference values during the whole study (Table 6).

Serum activities of ALP were significantly (p<0.05) decreased after treatment until reached their lowest activities at the 65th day when their values at days 15, 30 and 65 were compared with those at day 0 (Pretreatment). Serum activities of ALP during the experiment were within some reference ranges or higher than other reference values (Table 6).

**DISCUSSION**

The most common clinical findings of the examines sheep during the present work including appetite, mucous membranes, lymph nodes, temperature, pulse, respiration and rumen movement were within normal ranges reported by Radostits et al. (2007) and showed no abnormalities. The Capillary refill time (CRT) was < 2 S revealed normal viscosity of the blood with fluid and electrolytes balance. CRT was within the reference values reported by Jackson and Cockcroft (2002). The appetite and feed intake within the present work were normal, however, some articles reported that the common clinical signs of iodism in sheep were depression, loss of appetite, hyperthermia, cough, respiratory disturbances, and sometimes death were reported (McCauley et al., 1973). On the opposite hand, Clinical findings of iodine deficiency in sheep and goat included goitre, stunting growth, dropped milk yield, pregnancy toxemia, and reproductive abnormalities, including abortion, stillbirth, retained placenta, irregular estrus, infertility, depressed libido, and birth of small, weak, and hairless or short- and fuzzy-haired newborns (Whitlock et al., 2012).

A significant improvement in body weight gain was reported in treated sheep during the current study after treatment with KI. The highest body weight gain was reported at the 65th day. The same results were mentioned by Salem et al. (1999); Slippers et al. (2000) and might be attributable to addition of KI salts. Myers and Ross (1959) through comparative study on ewes mentioned that although only a very small body-weight change occurred as a result to the first T4 implant and iodine supplementation, comparing to the control, it was difficult to attribute this small change to any cause other than T4 administration, particularly when this body weight loss became more significantly after the second T4 treatment, and so when the sheep were grazing excellent pasture where an increase in body weight was observed in control sheep. The current study reported an improvement in body gain, however, there was a significant reduction in serum T3 and T4 that counteract with the fact that the reduction in plasma T4 concentrations correlates with reduced body gain (Aumont et al., 1989). The same results were reported by Herzig and Suchý (1996) who stated that the excessive amounts of iodine in farm animals were associated with reduction in feed intake and in turn a reduction in weight gain occurred.

Several factors appeared to affect the density and composition of the rumen ciliate protozoa. These factors related to the type and amount of feed consumed, frequency of feeding, rumen pH and turnover rate (Dehority, 1978;
The currently reported results stated that the density of rumen ciliate protozoa in investigated sheep was significantly improved at day 15 post-treatment with KI followed by a significant reduction at day 30 until reaching its lowest concentration at day 65. The concentrations of rumen ciliate protozoa in sheep throughout the present work were in agreement with that reported by Selim et al. (1996b); Baraka (2012); Gürelli et al. (2016) except for those at day 65 which were lower than these reference values. Khaled and Baraka (2011) also added that total protozoa ciliates count in sheep rumen showed gradual significant drop during the experiment and a remarkable change at 42 days in control and treated groups was clear. These changes might be attributable to the compensation of protozoa count to metabolic products of digestion and rumen pH.

Rumen constituents showed that rumen pH was significantly elevated after treatment with KI at days 15, 30 and 65 but they were generally within the reference range of rumen pH in sheep reported by Jasmin et al. (2011); Baraka (2012); Navarre et al. (2012). These changes were attributable to the addition of KI. Khaled and Baraka (2011) mentioned that rumen pH in both groups (Control and treated groups) of investigated sheep was significantly changed at day 21 and day 42, but generally within their reference range. On the other hand, several reports added that the control of rumen pH normally was a result of an interaction between food yields bases or buffers (Ammonia from degraded protein, non-protein nitrogen food), increasing rumen input of bases or buffers (Bicarbonates released from diet and saliva), absorption of rumen TVFAs and removing non-ionized VFAs in exchange with Bicarbonates (Maloj, 1972; Farid et al., 1979; Baraka, 2001).

TVFs were significantly elevated at the 15th day comparing to their concentrations at the 0 day. This remarkable improvement of TVFs continued in during the experiment at days 30 as well as it reached its highest values at day 65. The previous reported stated that TVFs were remarkably decrease in the 21st day followed by a significant elevation in last samples in control and treated sheep (Khaled and Baraka, 2011). The fluctuation in rumen TVFAs might be referred to that, the absorption rate of the volatile fatty acids depended on the pH of the abomasal fluid, when the pH declined below the physiological limit the rate of volatile fatty acids absorption increases (Dycker et al., 1994). TVFs along the current study were within the normal physiological range reported by McDonald et al. (2011). Many articles reported higher levels of TVFs in the rumen of sheep than those mentioned during this current work as reported by Machmüller et al. (2003); Akbar et al. (2009); Carro et al. (2009); Khaled and Baraka (2011).

Rumen ammonia levels were remarkably changed after supplementation of the examined sheep with KI as their levels were significantly dropped post-treatment starting from the 15th day comparing with ammonia values at day 0. This significant decrease in rumen ammonia concentration was more observable at the 30th and 65th days. Payne and Payne (1987) mentioned that rumen ammonia was absorbed to the liver for formation of urea and the urea to blood for BUN formation. Both of rumen urea and ammonia were used by the rumen bacteria for synthesis of bacterial protein, bicarbonates in the rumen are required for the carbon skeleton formation to induce the conversion of simple nitrogenous compounds into more complex molecules of bacterial protein. Visek (1972) mentioned that rumen urea concentrations were changed according to the rate of production and absorption of ammonia nitrogen, as well as on the rate of ammonia detoxification into urea in the liver. The present study reported that these levels of rumen ammonia were lower than that mentioned by Khaled and Baraka (2011), however they were higher than that mentioned by Rianto et al. (2005). In contrast, they were within the normal range according to other references such as to Satter and Slyter (1974); Akbar et al. (2009); McDonald et al. (2011). Khaled and Baraka (2011) concluded that concentrations of rumen ammonia in sheep were originated from the degradation of protein or non-protein nitrogen feed to ammonia and urea. McDonald et al. (2011) reported that 50 mg/L was the minimum concentration of rumen fluid ammonia for normal rumen microbial growth, and 85–300 mg/L is their optimum concentrations. The amount of feed protein entering the rumen was one of the factors that affect the lower concentrations of ruminal ammonia. Usually, high dietary protein content with high degradability will lead to an elevated ruminal fluid ammonia.

Differences in haematological and serum biochemical parameters in farm animals were related to genetics (breed), climatic conditions, gravidity period, stress, nutrition, rearing conditions and physical load, gender and age (Balikci et al., 2007; Tripathi et al., 2008). The present study revealed a potential impact of high intake of iodine in ewes on haematological and biochemical parameters of the blood of ewes where most of whole blood picture indices during present work including RBCs, Hb and PCV reported an improvement following treatment with KI except for MCHC and TLC that showed no remarkable changes. MCHC and TLC values were within the reference values mentioned by Radostits et al. (2000). RBCs were significantly increased at days 15 and 30 then they reached their highest values at day 65. RBCs values were lower than the reference values reported by Radostits et al. (2000); Aitken (2007) at days 0 and 15, however, they were within the normal range at the 30th day and the 65th day. Values of Hb, PCV, MCHC and TLC were agreed with what reported by Radostits et al. (2000); Jackson and
Iodine was an essential element that was used by the thyroid gland for thyroid hormones biosynthesis (Thilly et al., 1992). Through these hormones, iodine was included in controlling metabolism, growth and maturation of the cells, and the tissues development and growth (Hetzel 1989).

Thyroid hormones functions in the present study showed remarkable changes as a response to adding of KI to sheep diet where T3 and T4 showed significant reduction in their concentrations after treatment as well as this clear drop in serum levels of thyroid hormones reached their lowest values at day 65. Oei et al. (1983) mentioned that a decrease in serum T4 secretion rates might be due to an energy deficiency that might occur during pregnancy in the ewe. On the other hand, Peksa et al. (2013) reported in animal thyroid, acute inhibition of thyroidal organification of iodine by excess iodide was far from the acute inhibitory effect of excess iodide and changes in thyroid radiiodine uptake in hypophysectomized animals could be owned to the variations in dietary iodide intake which were obvious examples of autoregulation. Here, serum concentrations of T3 throughout the present study were higher than, however, serum T4 levels were within the normal reference ranges reported by Kozat et al. (2007); Badiei et al. (2009a); Novoselec et al. (2017) either pretreatment or post-treatment. In contrast, a study of the effects of excessive intake of iodine on thyroid functions revealed more clear results. Thyroid functions were not disturbed even after prolonged elevated iodine intake in common farm conditions (Convey et al., 1977; Hillman and Curtis, 1980). Furthermore, other reports stated equivalent findings in experimentally-induced iodism with the utilization of 200 up to 300 times higher dose than the daily requirement. The altered synthesis of thyroid hormones was observed only in calves, after long-term iodine intake in a dose 500 times more than their daily requirement (Leung et al., 1980). An inhibitory effect of excessive iodine intake on the thyroid hormones biosynthesis was observed in humans and animals (Nagataki, 1974) and horses (Baker and Lindsey, 1969). Nagataki (1974) thought that such inhibitory in-

S

Serum concentrations of electrolytes including sodium and potassium as well as blood levels of calcium and phosphorous were not significantly changed during the present study. They were within the normal physiological ranges mentioned by Radostits et al. (2000); Jackson and Cockcroft (2002). Blood levels of calcium showed slight increase while those of potassium showed slight decrease post-treatment. However, they were within the previously mentioned reference ranges. Darrell et al. (2012) mentioned the minerals which interfered with iodine absorption. They included rubidium, arsenic, fluorine, calcium, and potassium. Iodine appeared to be most available for use by the body during lactation and during winter season. The form in which iodine exists in the feed disturb availability as iodates were more readily absorbed than iodides. Serum levels of glucose were significantly increased after treatment particularly at days 30 and 65 when compared with their levels at day 0 (Pretreatment). The obtained results reported higher levels of glucose post-treatment than the reported reference values by Jackson and Cockcroft (2002); Aitken (2007). During iodine toxicosis some changes in biochemical indices were described as Hillman and Curtis (1980) reported significant improvement in serum glucose. However, Olson et al. (1984) did not report these elevations. In contrast, the excessive amounts of iodine in farm animals were associated with by hyperthermia, hypoglycaemia, a reduced milk production and reproduction disturbances (Huszenica et al., 2002).

BUN and creatinine concentration were decreased at days 15, 30 and 65 when compared with their values at day 0. The reduction in serum levels of BUN was significant, hence, it was not significant for serum creatinine. There was association between the reduction in both of rumen ammonia concentration and serum level of BUN where the two parameters were remarkably decreased. This could be explained as BUN concentrations were affected by ammonia absorption rates to the liver; Payne and Payne (1987) added also that ammonia was absorbed to the liver for formation of urea and the urea to blood for formation of BUN levels. Here, serum levels of BUN after treatment at day 15, 30 and 65, were lower than their reference values mentioned by Jackson and Cockcroft (2002), however, those of creatinine were higher than their reference values mentioned by Radostits et al. (2000) during the whole study. Hillman and Curtis (1980) referred to some changes in biochemical indices during iodism including significant

June 2021 | Volume 9 | Issue 6 | Page 852
advances in animal and veterinary sciences

Elevation in blood urea nitrogen.

The obtained results reported a remarkable reduction in serum activities of ALP after treatment until reached their lowest activities at the 65th day comparing with day 0. Serum activities of ALP during the experiment were within some reference ranges mentioned by Jackson and Cockcroft (2002) or higher than some other reference values reported by Aitken (2007). These results were in disagreement with Nudda et al. (2013); Dušová et al. (2014). Dušová et al. (2014) revealed that the blood urea concentrations and ALP activities were significantly higher in ewes of the experimental group (with the iodine intake of 5.1 mg per kg of dietary DM) than in ewes of the control group (with the iodine intake of 3.1 mg iodine per kg of dietary DM). The increased activity of blood ALP in ewes and lambs (The group with higher intake of iodine) was associated with a risk of an alteration in the thyroid activity. Therefore, Higher urea concentration and higher ALP activity in ewes and lambs with iodine intake above the upper limit of the permitted standard (EU standard, 2005) might confirm a potential risk of the thyroid activity changes.

CONCLUSION

Long-term 65 days KI supplementation produced variable significant changes in sheep mainly rumen metabolic functions, blood picture indices, thyroidal hormonal changes and body weight gain. This study will help the farms owners and breeders to use these feed additives to improve body gain through their role in improving rumen function and metabolic rates in sheep.

AUTHORS’ CONTRIBUTION

All authors have made substantial contributions to conception and design of the study. They have been involved in drafting, revising and final approval of the manuscript.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

• Akbar T, Ali MS, Golamreza Z, Maghsoud B, Adel A (2009). The study of diversity of ciliate protozoa in ghizel sheep fed in pasture and nourished by dried grape by-product. Am. J. Anim. Vet. Sci. 2: 37–41. https://doi.org/10.3844/ajavs.2009.37.41
• Aitken ID (2007). Diseases of Sheep. 4th ed. Blackwell Science Ltd, State Avenue, Ames, Iowa, USA. 601–60.
• Angelow L, Tsvetkova V, Jahreis G (2011). Influence of different selenium and iodine offer during the grazing period of sheep on the milk yield, milk performance and daily protein, fat and lactose secretion. Bulg. J. Agric. Sci. 17 (2): 139–144.
• Anke M, Groppel B, Bauch KH (1993). Iodine in the food chain. In. Delange JT and Glinoer G. (eds), Iodine Deficiency in Europe. Plenum Press, New York, USA. 151–157.
• Antunović Ž, Marić I, Matanić I, Senčić D, Šperanda M, Novoselec J (2009). Blood enzymes and thyroid hormone activity for the assessment of selenium supply in sheep (in Croatian). Krniva. 51: 131–137.
• Aumont G, Lamand M, Tressol JC (1989). Iodine nutrition in ewes: effects of low to high iodine intake on iodine content of biological fluids in pregnant and lactating ewes. Reprod. Nutr. Dev. 29 (1): 113–125. https://doi.org/10.1051/rnd:19890110
• Badiei K, Nikghadam P, Mostaghni K (2009a). Effect of cadmium on thyroid function in sheep. Comp. Clin. Pathol. 18: 255–259. https://doi.org/10.1007/s00080-008-0785-4
• Badiei K, Nikghadam P, Mostaghni K, Zarišić M (2009b). Effect of lead on thyroid function in sheep. Iran. J. Vet. Res. 10 (3): 28.
• Baker HJ, Lindsey JR (1969). Equine goiter due to excess dietary iodide. J. Am. Vet. Med. Assoc. 153: 1618–1630.
• Balicki E, Yildiz A, Gurdogan F (2007). Blood metabolite concentrations during pregnancy and post-partum in Akkaraman ewes. Small Rumin. Res. 67 (2–3): 247–251. https://doi.org/10.1016/j.smallrumres.2005.10.011
• Baraka TA (2001). Studies on blood electrolytes and acid-base balance of dromedary camels in health and disease. PhD (internal Medicine) Thesis, Faculty of Veterinary Medicine, Cairo University, Egypt, 180.
• Baraka TA (2012). Comparative Studies of Rumen pH, Total Protozoa Count, Generic and Species Composition of Ciliates in Camel, Buffalo, Cattle, Sheep and Goat in Egypt. J. Am. Sci. 8 (2): 448–460.
• Boland TM, Hayes L, Sweeney T, Callan JJ, Baird AW, Keely S, Crosby TF (2008). The effects of cobalt and iodine supplementation of the pregnant ewe diet on immunoglobulin G, vitamin E, T3 and T4 levels in the progeny. Animal. 2 (2): 197–206. https://doi.org/10.1017/S1751731108003844
• Carro MD, Ranilla MJ, Martí-n’Garci’ AI, Alcaide EM (2009). Comparison of microbial fermentation of high- and low-forage diets inRusitec, single-flow continuous-culture fermenters and sheep rumen. Animal. 4: 527–534. https://doi.org/10.1017/S1751731108003844
• Coles EH (1986). Veterinary Clinical Pathology. 4th ed. W.B. Saunders, Philadelphia, USA. 46–47, 132–139.
• Convey EM, Chapin LT, Kesner JS, Hillman D, Curtis AR (1977). Serum thyrotropin and thyroxine after thyrotropin releasing hormone in dairy cows fed varying amounts of iodine. J. Dairy Sci. 60: 975–980. https://doi.org/10.3168/jds.s0022-0302(77)83972-6
• Conway EF (1957). Microdiffusion analysis and volumetric errors. 2nd ed. Crosby- Lockwood and Son. Lt, London, UK.
• Cottyné BS, Boucq ChV (1968). Rapid method for the gas chromatographic determination of volatile fatty acids in...
Advances in Animal and Veterinary Sciences

Darrell L, Rankins JR, Pugh DG (2012). Feeding and Dehority BA (1978). Specificity of rumen ciliate protozoa in cattle and sheep. The Journal of Protozoology. 25: 509–513. https://doi.org/10.1111/j.1550-7408.1978.tb04177.x

Dehority BA (1984). Evaluation of subsampling and fixation procedures used for counting rumen protozoa. Appl. Environ. Microbiol. 48: 182–5.

Dušová H, Travníček J, Peksa, Šimák-Libalová ZK, Šimíková KA, Falta D, Švéjcová K (2014). The influence of high iodine intake on chosen blood parameters of sheep. actauniversitatis agriculturae et silvicultrae mendelianae brunensis. 62: 71-79.

Dycker CH, Lechner-Doll M, Hoffrogge P, Engelhardt WV (1994). Short chain fatty acid absorption from the forestomach in camels. J. Camel Pract. Res. 108. 110.

EU standard (2005). Commission Regulation /EC/No. 183/2005 of 8 September amending the conditions for authorization of a number of feed additives belonging to the group of trace elements. OEUJ. 233: 8–10.

Farid MFA, Shawkat SM, Abdel-Rahman MH (1979). Franzolin R, Dehority BA (1996). Effect of prolonged high-concentrate feeding on ruminal protozoa concentrations. J. Anim. Sci. 74: 2803–9. https://doi.org/10.2527/1996.74112803x

Gürelli G (2014a). First record of Diplodinium rangiferi Dogiel, 1925 (Ophryoscolecidae, Entodiniomorphida) from domestic cattle. Turk. J. Zool. 38: 369–72.

Gürelli G (2014b). Rumen ciliate fauna (Ciliophora, Protista) of Turkish domestic goats living in ‘Izmir, Turkey. Turk. J. Zool. 38: 136–43.

Gürelli G, Iro A (2014). Intestinal ciliated protozoa of the Asian elephant Elephas maximus Linnaeus, 1758 with the description of Triplumaria izmirae new species. Eur. J. Protistol. 50: 25–32. https://doi.org/10.1016/j.ejop.2013.10.002

Gürelli G, Canbulat S, Aldayarov N, Dehority BA (2016). Rumen ciliate protozoa of domestic sheep (Ovis aries) and goat (Capra aegagrus hircus) in Kyrgyzstan. FEMS Microbial. Lett_363 (6):1-7. https://doi.org/10.1093/femsle/fnw028

Harvey JH (2001). Atlas of Veterinary Hematology. Elsevier, WB Saunders Company, Philadelphia, Pennsylvania, USA. 3-74.

Herzeg I, Suchý P (1996). Actual experience of importance iodine for animals. Vet. Med. – Czech. 41: 379–386.

Herzeg I, Pisaříková B, Kursa J, Suchý P (2000). Utilisation of iodine from different sources in pigs. Arch. Anim. Nutr. 53: 179–189. https://doi.org/10.1080/1745039009381945

Hetzel BS (1989). The Story of Iodine Deficiency: An International Challenge in Nutrition. Oxford University Press, Oxford, UK.

Hillman D, Curtis AR (1980). Chronic iodine toxicity in dairy cattle: Blood chemistry, leukocytes, and milk iodine. J. Dairy Sci. 63: 55–63. https://doi.org/10.3168/jds.S0022-0302(80)82887-6

Huszenica GY, Kulcsár M, Rudas P (2002). Clinical endocrinology of thyroid gland in ruminants. Vet. Med. – Czech. 47 (?): 199–200.

Jackson PGG, Cockcroft PD (2002). Clinical Examination of Farm Animals. Blackwell Science Ltd, State Avenue, Ames, Iowa, USA. 219-247, 301-305.

Jasmin BH, Boston RC, Modesto RB, Schaer TP (2011). Perioperative Ruminal pH Changes in Domestic Sheep (Ovis aries) Housed in a Biomedical Research Setting. J.A.A.L.A.S. 50 (1): 27-32.

Khaled NF, Barak TA (2011). Influence of TOMOKO* (Direct-Fed Microbials) on Productive Performance, Selected Rumen and Blood Constituents in Barky Finishing Lambs. J. Am. Sci.7 (9): 564-570.

Kirschmann G, Kirschmann JD (1996). Nutrition almanac. 4th ed. McGraw Hill, New York, USA. 494.

Kozat S, Mert H, Yuksel N, Mert N, Ekin S (2007). Serum levels of some trace elements and thyroid hormones in yearling rams with retardation in growth. Bull Vet. Inst. Pulawy. 51: 117-120.

Latimer KS, Mahaffey EA, Prasse KW (2011). Duncan and Prasse’s Veterinary Laboratory Medicine: Clinical Pathology. 5th ed. Blackwell Publishing Ltd, Wiley-Blackwell, Iowa, USA. 3–82.

Leirer R, Körber R, Jäkel L (1983). Untersuchungen zum Jodmangelsyndrom beim Rind. Monatsh Vet. Vierähr. 38: 103–109.

Leung K, Convey EM, Conner GH (1980). Effect of dietary iodide on hypothyseal and thyroid hormone secretions in Holstein-Friesian heifers. Am. J. Vet Res. 41: 1402–1407.

Machmüller A, Carla RS, Michael K (2003). Effect of coconut oil and defaunation treatment on methanogenesis in sheep. Reprod. Nutr. Dev. 43: 41–55. https://doi.org/10.1051/rd:2003005

Maloy GMO (1972). Comparative studies on digestion and fermentation rate in the fore stomach of the one humped camel and the zebu steer. Res. Vet. Sci.13: 476–481.

McCauley EH, Linn JB, Goodrich RD (1973). Experimentally induced iodide toxicosis in lambs. Am. J. Vet. Res. 34: 65-70.

McDonald P, Edwards RA, Greenhalgh JFD, Morgan CA, Sinclair LA, Wilkinson RG (2011). Animal Nutrition. 7th ed. Pearson, Prentice Hall, United Kingdom.

Medrano F, Rodolfo, Hua HJ (2016). Advances in Thyroid Hormones Function Relate to Animal Nutrition. Annals of Thyroid Research. 2 (1): 45–52.

Myers BJ, Ross DA (1959). The effects of iodine and thyroxine administration on the Romney Crossbred ewe. New Zealand J. Agric. Res. 2 (3): 552–574. https://doi.org/10.1080/00288233.1959.10418035

Nabeel B, Kadum NB, Oday K, Lubaibi OK (2017). Clinical study hypothyroidism in goats and treatment by iodine compounds. J. Entomol. Zool. Stud. 5 (4): 1956-1961.

Nagataski S (1974). Effect of excess quantities of iodide. In. Greer MA and Solomon HD (eds), Handbook of physiology, Sect 7, Endocrinology, 3 eds American Physiological Society. Washington DC, USA. 329.

Nagy DW, Pugh DG (2012). Handling and Examining Sheep and Goats. In. Pugh DG and Baird AN (eds), Sheep and goat medicine. 2nd ed. Saunders Elsevier, Philadelphia, USA. 1–17.

Navarre CB, Baird AN, Pugh DG (2012). Diseases of the Gastrointestinal System. In. Pugh DG and Baird AN (eds), Sheep and goat medicine. 2nd ed. Saunders Elsevier, Philadelphia, USA. 71–76.

Novoselec J, Speranda M, Klír Z, Mioč B, Steiner Z, Antunović...
Z (2017). Blood biochemical indicators and concentration of thyroid hormones in heavily pregnant and lactating ewes depending on selenium supplementation. Acta Vet. Brno. 86: 353–363. https://doi.org/10.2754/avb201786040353

• NRC-National Research Council (1985). Nutrient requirements of sheep. 6th ed. National Academy Press, 2101 Constitution Avenue, NW, Washington, D.C. 20418, USA.

• NRC-National Research Council (2001). Nutrient requirements of dairy cattle. 7th ed. National Academies Press, Washington, D.C., USA. 266-267.

• Nudda A, Battacone G, Bomboi G, Floris B, Decandia M, Pulina G (2012). Effect of dietary iodine on thyroid hormones and energy blood metabolites in lactating goats. Animal. 7 (1): 60–65. https://doi.org/10.1017/S1751731112001073

• Oci T, Chulk A, Burke V, Nordschow C, Lemons JA, Jansen RD, Schreiner RL (1983). Effect of maternal fasting on fetal T4 concentration in the sheep. Nutr. Rep. Int. 27: 231–237.

• Ogimoto K, Imai S (1981). Atlas of Rumen Microbiology. Japan Scientific Societies Press, Tokyo, Japan.

• Olson WG, Stevens JB, Anderson J, Haggard DW (1984). Iodine toxicosis in six herds of dairy cattle. J. Am. Vet. Med. Assoc. 184: 179–181.

• Ong CB, Herdt TH, Fitzgerald SD (2014). Hyperplastic goiter in two adult dairy cows. J. Vet. Diagn. Invest. 26 (6): 810–814. https://doi.org/10.1177/1040638714554441

• Payne JM, Payne S (1987). The metabolic profile test. Oxford: University Press, Oxford, UK. 178.

• Peksa Z, Trávníček J, Konečný R, Jelínek F, Dušová H, Hasoňová L, Pálka V (2013). Histometric and biochemical properties of the thyroid gland in sheep with high iodine supplementation. Acta Vet. Brno. 405–409. https://doi.org/10.2754/avb201382040405

• Pugh DG, Baird AN (2012). Sheep and goat medicine. 2nd ed. Saunders Elsevier, Philadelphia, USA.

• Radostits OM, Gay CC, Blood DC, Hinchcliff KW (2000). Veterinary Medicine. 9th ed. W.B. Saunders, London, UK. 1819–1822.

• Radostits OM, Gay CC, Hinchcliff KW, Constable PD (2007). Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats. 10th ed. Saunders Elsevier, Philadelphia, USA. 3-37.

• Rianto E, Thwaites CJ, Nolan JV (2005). The effect of ambient temperature and water availability on feed digestibility, rumen microbial nitrogen production, and rumen condition in sheep. Bulletin of Animal Science. 29 (1): 10–18.

• Salem IA, Dagash HA, Kobeisy MA, El-Tarawy AAM (1990). Effect of supplemented dietary iodine on growth performance and thyroid gland activity in Egypt in Saeedi lambs. Assiut Vet. Med. J. 4 (81): 1999.

• Satter LD, Slyter LL (1974). Effect of ammonia concentration of rumen microbial protein production in vitro. Br. J. Nutr. 69: 2755–2766. https://doi.org/10.1079/bjn19740073

• Selim HM, Imai O, Yamato O, Miyayawa E, Maede Y (1996a). Ciliate protozoa in the forestomach of the dromedary camel, (Camelus dromedarius), in Egypt, with description of a new species. J. Vet. Med. Sci. 9: 833–837. https://doi.org/10.1292/jvms.58.833

• Selim HM, Imai O, Yamato O, El Kabbany A, Kiroloss F, Maede Y (1996b). Comparative study of rumen ciliates in buffalo, cattle and sheep in Egypt. J. Vet. Med. Sci. 58 (8): 799-801. https://doi.org/10.1292/jvms.58.799.

• Slippers SC, Letty BA, Villiers JF (2000). Prediction of the body weight of Nguni sheep. S. Afr. J. Anim. Sci. 30 (1): 128. https://doi.org/10.4314/sajas.v30i4.3940

• Thilly CH, Vanderpas JB, Bebe N, Ntambue K, Contempre B, Swennen B, Moreno-Reyes R, Bourdoux P, Delange F (1992). Iodine deficiency, other trace elements and goitrogenic factors in the etiopathogeny of iodine deficiency disorders (IDD). Biol. Trace. Elem. Res. 32: 229–243. https://doi.org/10.1007/BF02784606.

• Travnické J, Kroupová V, Koncny R, Srťová M, Šťastná J, Hasenova L, Mikulová M (2010). Iodine status in ewes with the intake of iodine enriched alga Chlorella. Czech J. Anim. Sci. 55 (2): 58–65. https://doi.org/10.17221/40/2009-CJAS

• Tripathi MK, Mondal D, Karim SA (2008). Growth, haematology, blood constituents and immunological status of lambs fed graded levels of animal feed grade damaged wheat as substitute of maize. J. Anim. Physiol. Anim. Nutr. (Berl). 92 (1): 75–85. https://doi.org/10.1111/j.1439-0396.2007.00712.x

• Visick WJ (1972). Effects of urea hydrolysis on cell life-span and metabolism. Fed. Proc. 31:1178–1193.

• Warner ACI (1964). Production of volatile fatty acids in the rumen: methods of measurements. Nutr. Abstr. Rev. 34: 339-352.

• Whitlock BK, Coffman EA, Pugh DG (2012). Diseases of the Endocrine System. In. Pugh DG and Baird AN (eds), Sheep and goat medicine. 2nd ed. Saunders Elsevier, Philadelphia, USA. 231-255.

• Zapletal O (1967). The toxicity of urea and possibility its influence at cattle. PhD Thesis, Brno, Czech Republic. 120.