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Effect of broccoli stem and leaves on some biochemical and hormonal parameters in Kurdi (Karadi) ewes

Nawzad Muhammed Aziz[1]*, Shagul Jalal Mohammed[2], Bahroz Muhammed Saleh[2]

1. Department of Animal Resources, College of Agricultural Engineering Sciences, University of Salahaddin, Kurdistan Region, Iraq
2. Department of Animal Science, College of Agricultural Engineering Sciences, University of Sulaimani, Kurdistan Region, Iraq

** Corresponding author's Email: Nawzad.aziz@su.edu.krd*

ABSTRACT

This study was conducted to determine the effects of adding varying amounts of broccoli waste (by-product) to the ration in ewes' diets based on some biochemical and hormonal markers in Kurdi ewes, which is the first of its kind in Iraq. Twelve Kurdi (Karadi) ewes aged 2-3 years were divided into three groups at random to ensure a non-significant difference between the groups. Each group had four ewes with an average body weight of 62.75 kg. T_1 (no broccoli wastes), T_2 and T_3 treatments fed (250 g and 750 g broccoli wastes were introduced in the first and second months) and (500 g and 1000 g of broccoli wastes were added in the first and second months) respectively. At the end of the trial, about 2 mL of blood was obtained from each animal's jugular vein before morning feeding. According to our results, lipid profile of the animals increased in BB fed animals compared to control group. Although TG and VLDL showed a decrease in the second treatment compared to control group. Concentrations of glucose, total protein, albumin, globulin, ALT, AST, creatinine, triiodothyronine and thyroxin hormone, did not change significantly ($p > 0.05$), while insulin hormone and urea showed a significant change between the BB treated groups compared to control group ($p < 0.05$). The ewes in our experiment fed by-product (BB) showed no clinical signs of glucosinolate toxicity because the concentration was low and there were no symptoms of toxicity.

Keywords: Broccoli, by-products, Biochemical parameters, ruminants, Thyroid hormones, and Brassica vegetable.
Article type: Research Article.

INTRODUCTION

With a population of 3,500,000 sheep in Kurdistan, growing sheep is an important contributor to the agricultural economy (Animal Production and Veterinary Directorate 2011). The overall number of sheep in towns, pastures, and animal raising farms in 2017 was 2,399,176, according to KRSO data. It is critical to use non-traditional feed ingredients to address the protein shortage problem by reducing feeding expenses, especially given the current rising cost of protein feed ingredients (Mahmoud 2016). Agri-food by-products, whether from field stubbles or the canning industry, provide an alternative source of forage for livestock, letting for the use of local resources while lowering feed costs without compromising animal performance or productivity, as long as the rations that include these feeds are controlled. Using these by-products can also help to reduce the amount of waste generated by the agricultural food producing business, reducing disposal costs and declining the harmful gas emissions produced by unregulated fermentation of agricultural wastes. Furthermore, by reducing the amount of land and resources dedicated to the production of livestock feed, using agro-food wastes benefits the value chain (Monllor *et al.* 2020). The increasing frequency and intensity of droughts in the world have led to shortages of common livestock feedstuffs (Alipour & Rouzbehan 2010). Alternative feed sources, including agricultural waste by-

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products, need to be investigated as ingredients for livestock diets. Broccoli by-product (BB) comprised of stem and leaves could be an alternative. By using by-products for ruminant feeding, high-quality food for humans, such as meat and milk, can be obtained (Wrick *et al.* 1983). Broccoli by-product (BB), which consists of stems and leaves from broccoli, might be replaced. After collecting the broccoli blossom bud for human consumption, about two-thirds of the plant (outer leaves and stems) is left in the field (Partovi *et al.* 2020). Campas-Baypoli *et al.* (2009) proposed a method for utilizing field-produced broccoli wastes, which account for the largest proportion of vegetable and provide a management and disposal challenge for farmers. According to the Food and Agriculture Organization of the United Nations (FAO), 25,984,758 tons of broccolis were harvested worldwide in 2017. Broccoli production has risen by 32.1% in the last decade, reaching 37.2106 tons in 2018 (Values of production combined with cauliflowers). China and India are the top two manufacturers. (Faostat 2020). Broccoli production has expanded tremendously as a result of its relevance as a health-promoting food. Broccoli intake has been shown to be useful in the prevention of chronic diseases due to its high concentration of bioactive phytochemicals (glucosinolates, isothiocyanates, and phenolic compounds) and nutrients (vitamins and minerals; Domínguez-Perles 2010). During agricultural production (cultivation and harvesting), processing, transportation, and consumption in the broccoli supply chain for human consumption, several wastes of vegetable material occur (De Evan *et al.* 2019). As BB are high in water and perish fast, adding them in ruminant diets could reduce farming costs and pollution caused by their accumulation. Different research have looked at using BB as feed ingredients in the diets of dairy cows (Yi *et al.* 2015), sheep (Mahmoud 2016), goats (Panwar 2017), and rearing lambs (Patrovi *et al.* 2020), and all have found positive outcomes, offering tremendous nutritional potential for ruminants, according to Evan *et al.* (2020), however, their practical use is limited due to their high water. Fresh BB has a high crude protein (CP; 270 g kg^{-1} DM) content and a low neutral detergent dietary fibre (NDF; 280 g kg^{-1} DM) and 9.87 MJ/kg DM in terms of metabolizable energy (Hu *et al.* 2011). Its organic matter (OM) digestibility *in vivo* was 86.9% (Wadhwa *et al.* 2006). According to previous studies, 70% of the total weight of broccoli plants is squandered in the field, while 45–50% of the harvested edible broccoli florets are wasted during processing and transportation (Campas-Baypoli *et al.* 2009). As a result, this study was conducted to determine the effects of adding varying amounts of broccoli waste to the ration in ewes' diets based on some biochemical and hormonal markers in Kurdi ewes, which is the first of its kind in Iraq.

MATERIALS AND METHODS

The research was conducted at the University of Salahaddin's College of Agricultural Engineering Sciences on the farms of the Iraqi governorate of Erbil's Animal Resources Department (Grdarash). Twelve Kurdi (Karadi) ewes aged 2-3 years were divided into three groups at random to ensure a non-significant difference between the groups. Each group had four ewes with an average body weight of 62.75 kg. The first group was T_1 (no broccoli wastes), whereas the second group T_2 (250 g and 750 g broccoli wastes were introduced in the first and second months, respectively), and the third group T_3 (500 g and 1000 g were added in the first and second months, respectively). In each group, the daily amount of feed intake (feed concentrate) was 8 kg (2 kg for each individual) for two months before the experiment, then 10 kg (2.5 kg for each individual) for the first and second months until the end of the trial. Table 1 contains the feed sample analysis report, and Table 2 the feed concentrate analysis.

Collection of blood samples and plasma

At the end of the trial, about 2 mL blood was obtained from each animal's jugular vein before morning feeding (from all ewes in each group) in clean sterile tubes and transferred to a vacutainer for serum preparation. Centrifugation was done at 3000 r.p.m for 15 minutes and then the plasma was separated and stored at -20 °C until glucose, cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), triglycerides, total protein (TP), serum albumin, serum globulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) were measured using a reagent kit (PZ CORMAY S.A., Poland) and reagent kit with Auto Chemistry Analyzer (Model Polimak M10/2, Italy). Furthermore, utilizing Cobas e 411 automated analyzers, biochemical analysis was performed to evaluate Insulin, T3 and T4 (nmol L^{-1}) concentrations using the Electrochemiluminescence immunoassay (ECLIA) method. A commercially available kit was used (Roche Diagnostic GmbH, and Mannheim, Germany).

Statistics

Table 1. Sample analysis report.

References	Results (%)	Tests
IR 600 (KJELDAHL)	17.6	Protein
ISO 598	4.7	Ash
By Oven (Memmert)	8.64	Moisture
ISO 6492 EEG (SOXELETT)	0.7	Fat
ISO 5498/1981	6.3	Fibre

Table 2. Feed concentrate analysis.

Compositions	Results (%)
Barley	50
Bran	28
Corn	10
Soybean	10
Lime	1
NaCl	1

To compare differences in component levels with the control group, the data were assessed using standard statistical procedures done with XLSTAT-PRO (version 7.5), including one-way analyses of variance (ANOVA) and repeated assessments. The least squares method was used to display the data (mean ± SD). In addition, $p < 0.05$ was used as the significance level. Duncan's test was used to compare the treatment groups' mean values.

RESULT AND DISCUSSION

Table 3. Serum lipid profile.

Parameters	T_1 (Control)	T_2	T_3
Cholesterol (mg dL^{-1})	57 ± 06	68.5 ± 0.096	63 ± 0.16
TG (mg dL^{-1})	17.25 ± 0.397	16 ± 0.26	18 ± 0.297
HDL (mg dL^{-1})	38.325 ± 0.14	40.4 ± 0.13	40.91 ± 0.12
LDL (mg dL^{-1})	13.2 ± 0.38	17.15 ± 0.11	19.525 ± 0.45
VLDL) mg dL^{-1})	3.45 ± 0.397	3.2 ± 0.26	3.6 ± 0.297

Table 3 illustrated that the blood levels of Cholesterol, Triglyceride, HDL, LDL and VLDL, were not affected by treatments ($P < 0.05$). Although there was an increased cholesterol and LDL levels in the second and third treatments (T_2 and T_3) that can be seen clearly. In addition, all parameters are increased in the third treatment. According to the results, lipid profile of the animals increased in the BB-fed animals compared to control group. Although TG and VLDL showed a decrease in T_2 compared to control group. Our results are in disagreement with those obtained by Cox-Ganser *et al.* (1994) who reported that transfer of sheep from grazing grass to Brassica forage results in a reduction in plasma cholesterol, while in agreement with Mahmoud (2016) that cholesterol increased when lambs fed broccoli by-products at 20 and 40% levels in replacing of Berseem hay (BH), respectively. Human intervention studies have shown that cruciferous vegetable-rich diets can affect plasma lipid and cholesterol profiles (Aiso *et al.* 2014). According to Charlotte *et al.* (2015), participants who consumed 400 g/week of high glucoraphanin (HG) broccoli over a period of 12 weeks, exhibited significantly lower plasma low density lipoprotein-cholesterol (LDL-c) levels than those who consumed 400 g/week conventional broccoli. However, HDL, total cholesterol, and triglyceride levels did not alter significantly. In contrast to our results, Diabetic rats treated with hydro-alcoholic extract of broccoli leaves showed a decrease in serum contents of total cholesterol (TC), LDL, TG and VLDL, although our results showed a decline in both VLDL and TG in T_2, albeit, similar to our results, a significant increase in insulin level ($p < 0.05$). The difference between our results with theirs may be due to the effect of presence of flavonoids and their antioxidant features. Glycosinolate paper leaves were higher in carotenoids, chlorophylls, vitamins E and K, total phenolic content, and antioxidant activity that are higher compared to other tissues. This is in turn due to high level of anti-oxidative substances existing in broccoli leaves, which can induce desirable metabolic changes associated with hepatic enzymes as to improve undesirable changes blood glucose and lipid levels blood thyroid hormones increased and cholesterol reduced in sheep grazing brassica, according to Cox-Ganser *et al.* (1994). They reported that, thyroid hormones and blood cholesterol have an immediate inverse correlation. This effect was not detected in the present study. As a result, antinutritional elements in BB are unlikely to have played a substantial influence at the current level of dietary uptake. Overall, our assessment of broccoli's effect on lipid profiles differs from earlier studies that found a decrease in plasma triglycerides, LDL cholesterol, and total cholesterol according to Murashima *et al.* (2004) and Lee *et al.* (2004). The explanation for these discrepancies could be due to the different metabolic models used, as well as our use of broccoli as a product in our treatments versus broccoli sprouts in theirs, despite the fact that most of the nutrients are the same but at different quantities.

Table 4. Thyroid hormones (T3, triiodothyronine, and T4, thyroxine), hepatic enzymes including aspartate aminotransferase (AST) and alanine aminotransferase (ALT), total protein (TP), serum albumin and globulin.

Parameters	**T_1 (Control)**	**T2**	**T3**
ALT (U L^{-1})	19.975 ± 0.42	15.9 ± 0.25	18.7 ± 0.14
AST (U L^{-1})	77.3 ± 0.18	92.575 ± 0.19	101.95 ± 0.23
Tot protein(g dL^{-1})	6.46 ± 0.049	7.14 ± 0.064	6.85 ± 0.083
Albumin(g dL^{-1})	3.045 ± 0.26	3.433 ± 0.08	3.465 ± 0.06
Globulin(g dL^{-1})	3.415 ± 0.27	3.705 ± 0.17	3.383 ± 0.18
Insulin (µU dL^{-1})	3.683 ± 0.45^{ab}	1.369 ± 0.499^{b}	4.42 ± 0.28^{a}
Glucose(mg dL^{-1})	54.067 ± 0.10	53.467 ± 0.01	58.025 ± 0.06
Urea (mg dL^{-1})	20.525 ± 0.08^{b}	28.35 ± 0.2^{a}	28.325 ± 0.10^{a}
Creatinine (mg dL^{-1})	0.745 ± 0.15	0.725 ± 0.13	0.7725 ± 0.12
T3 (nmol L^{-1})	3.07 ± 0.22	2.618 ± 0.096	3.158 ± 0.22
T4 (nmol L^{-1})	92.448 ± 0.18	106.775 ± 0.07	98.18 ± 0.21

Note: * Different letter in the same row within the same group mean significant differences ($P < 0.05$), absent of letters mean no significant difference between treatments.

Table 4 depicted that the blood levels of glucose, total protein, albumin, globulin, ALT, AST, creatinine, triiodothyronine and thyroxin hormone, did not change significantly ($p < 0.05$), while insulin hormone and urea showed a significant change between the BB-treated groups compared to control group ($p < 0.05$). Blood parameter concentrations are an integrated index of nutrient supply in relation to nutrient use, which is a self-governing feature of the animal's physiological condition and provides an immediate indication of nutritional status at a certain point in time (Pambu-Gollah *et al.* 2000). The lack of variations in blood biochemistry measures between the treatments in this investigation was most likely due to similar nutritional intakes and digestibility. The serum glucose, total protein, albumin, globulin, AST, ALT, and urea values were all within acceptable ranges for sheep in all treatments (Radostits *et al.* 2007). Total protein, albumin, and globulin concentrations were found to be within the normal ranges in previous studies (Kaisar Rahman *et al.* 2018; Borjesson *et al.* 2022). Since the liver is the primary organ for albumin synthesis, identical blood albumin values among the ewes suggested appropriate liver function. The albumin and globulin levels in the serum of the lambs in this investigation suggest that they were free of any health issues that could have hampered their performance (Radostits *et al.* 2007). In the present study, albumin levels were higher in T_2 than in T_1 and also in the control. Mahmoud (2016) observed the same results when using broccoli wastes in sheep rations. However, in contrast to our results they observed that, animals fed higher percentage of broccoli by-product showed the highest total protein level, although we found the highest in T_2. Albeit, ewes in T_2 recorded the highest globulin and total cholesterol compared to T_1 (control). Blood plasma total protein and its fractions can be utilized as indicators to assess ruminant nutritional status and physiological change, according to Kummer *et al.* (1981). So, the elevated globulin production by the liver could reflect a good hepatic function of these animals and correlates very well with their high immunity status (Grimimger 1986). In line with this, noteworthy, supplementing cow diets with various levels of natural feed additives exhibited a good impact on their immunity state, as measured by globulin level rising. Also Craig (1999) clarified albumin as one of the major proteins that maintains the blood's osmotic pressure. The ability of animals to maintain reserve proteins after their bodies have reached maximum depositing tissue capacity is reflected in albumin and globulin values (Stroev 1989). Total protein (Table 4) were within the acceptable ranges for ewes for all treatments (Desco *et al.* 1989). However, according to Doornenbal *et al.* (1988) blood parameter has low correlation with the actual animal protein status. Conversely, blood albumin (Table 4) is generally considered with urea as an indicator of the animal protein status. High circulating globulins are found generally when an animal immune system is challenged. In the present study (Table 4), this blood parameter was in greater concentrations in T_2 compared to other groups. In both the control and T_3 groups, ewes exhibited decreased blood concentrations of circulating globulins, indicating that the immune system was not overworked. Animal health may be harmed as a result of transportation (Schwartzkopf-Genswein *et al.* 2010). Kelley *et al.* (1982) concluded that heat and cold stress can drive either antibody or cell-mediated immunity in dairy calves. Carr *et al.* (1992) reported that cold-stressed mice produce more antibodies which also in agreement with Hicks *et al.* (1998). In cold-stressed pigs, there was an increase in natural killer cytotoxicity (Hangalapura *et al.* 2004). The effect of cold stress on lymphocyte proliferation in chickens was found to be enhanced (Miller & McConnell (2012). Antibody production was shown to be higher in chronically malnourished new-borns, implying an adaptive protective complex against infectious illness and mortality (Kaneko *et al.* 1997). Over time, total protein levels rise, albumin levels fall, and globulin levels rise in all animals (Forstner 1968; Tumbleson *et al.* 1972). Plasma proteins are affected by dietary changes, though the changes are usually small and difficult to detect and interpret. Albumin levels increase in both BB-fed treatments and broccoli leaves contain high amounts of carotenoid, according to Liu *et al.* (2018). In cows, there was a direct link between vitamin A and reduced albumin levels (Erwin *et al.* 1959), which was corrected by the administration of carotene. In rats, a severe protein shortage leads to hypo-proteinemia and hypoalbuminemia (Weimer 1961), chickens (Leveille & Sauberlich 1961), and dogs (Allison 1957). All values remained in the normal range reported for triiodothyronine (T3, Alemu 1977) and thyroxin (T4; Ruckebush *et al.* 1991). Anti-nutritional S-methylcysteine sulfoxide and glucosinolates found in brassicas have been documented to be goitrogenic, affecting the formation of T3 and T4 (Chorfi *et al.* 2015). As it is known, one of the most prevalent signs of glucosinolate exposure is thyroid dysfunction, which leads to endocrine gland enlargement (goitre). Impairment of growth, reduced feed conversion, and impairment of development, fertility, and reproduction are all symptoms of hypothyroidism after exposure to hazardous amounts of glucosinolate (Mawson *et al.* 1994a, b; Mithen *et al.* 2000; Burel *et al.* 2001; Conaway *et al.* 2002). The ewes in our experiment fed BB showed no clinical signs of glucosinolate toxicity, since the glucosinolate level was low and there were no symptoms of toxicity (EFSA 2008). Thyroid gland function and thyroid hormone (TH) activity, according to Todini (2007), are crucial in maintaining productive performance in domestic animals (growth, milk, and hair fibre production), and circulating TH can be used as indicators of the animals' metabolic and nutritional status (Riis & Madsen 1985). Thyroid hormones are involved in nutrient absorption, metabolism, and calorigenesis (Todini *et al.* 2007), growth and development (Nixon *et al.* 1988), and reproduction (Blaszczyk *et al.* 2004). Thyroid hormones play an important role in nutritional absorption, metabolism, and calorigenesis (Todini *et al.* 2007), as well as reproduction (Blaszczyk *et al.* 2004), along with growth and development (Nixon *et al.* 1988). The explanation for these differences could be due to the different metabolic models applied, as well as our use of broccoli by-products in our treatments versus broccoli sprouts in theirs, despite the fact that most of the phytonutrients are the same but at different quantities (Liu *et al.* 2018). Broccoli is a well-known abundant source of glucosinolates, chemical compounds with a potential of anti-thyroid effect on thyroid function (Felker *et al.* 2016). Our results did not display any significant alterations in T3 and T4, as we fed BB to ewes. Similar to our results, an experiment was carried out by Pasko *et al.* (2018) reporting that both levels of T3 and T4 remained unchanged following broccoli sprouts ingestion. Insulin hormone levels differed significantly between the BB-treated and control groups ($p < 0.05$). Insulin also functions as the strong energy status predictors when used in conjunction with other blood parameters (Caldeira *et al.*2007). In conjunction with insulin, which has a close relationship with the nutritional state of the animal, the level of glycaemia could aid in the diagnosis of energy status (McCann *et al.* 1992; Delavaud *et al.* 2002; Calde ira *et al.* 2007). On the other hand, high quantities of free fatty acids usually imply a negative energy balance (Delavaud *et al.* 2002; Caldeira *et al.* 2007), while conversely, low levels indicate a positive one (Caldeira *et al.* 2007). Insulin affects glucose metabolism in liver cells, the major organ of glucose homeostasis, albeit in a somewhat different focus (Kaneko *et al.* 1997). The study by Farahmandi *et al.* (2013) implies that treating with hydro-alcoholic extract of broccoli leaves leaf exhibits the blood glucose and lipid decreasing effects on rats affected by diabetes. These effects can be due to the presence of flavonoids and their antioxidant features. In the present study, treatments displayed little effect on blood glucose levels, though was elevated in the third treatment (T_3) and were within the usual range for ewes (Kaneko *et al.* 1997; Meyer 1998; Dias *et al.* 2010). Except for the control group, blood glucose levels tended to rise or stay steady for the other two treatments. Blood glucose levels have been linked to body weight (Patil 1979). Thermal stress has also been linked to lower blood glucose levels (Gwaze *et al.* 2010). When compared to the control group, blood urea levels increased considerably in both BB-fed groups (Table 4). The amount of urea in the blood is proportional to the amount of crude protein (CP) in the forage (Wilson *et al.* 2010). Crude protein is abundant in fresh BB (CP; 270 g kg^{-1} DM; Wadhwa *et al.* 2006). Similar to our findings, Patrovi *et al.* (2020) observed an elevation in the plasma urea in both treatments with added BBWS. However, in contrast to our findings Chorfi *et al.* (2015) reported a declined-urea in calve groups fed kale, a brassica family member, similar to broccoli and high in glucosinolates. All of the liver enzymes we examined were within the usual permissible range for the bovine species (Kaneko 2008). The AST and ALT levels are indicators of a liver reaction to low-quality feed, toxins, or anti-nutritional factors. Broccoli and its by-products (BB) contain glucosinolates and S-methylcysteine sulfoxide, which have been shown to impact the liver and boost hepatic enzymes (Stoewsand 1995; Leu *et al.* 2018). There was an increase on one of the hepatic enzymes. The liver enzyme (AST) increased in both BB-treated animals. It is generated in the cytosol and mitochondria of cells, and its levels rise when hepatocytes are damaged. In our study, it stayed within the reference value interval for healthy animals, indicating that the broccoli wastes provided to the animals were of good quality. Our results show that, blood albumin levels were lower in control group, and increased in other BB-fed treatments. In addition, as the urea levels in both T_2 and T_3 upraised, the protein status was influenced. The greater urea levels in T_2 and T_3 at the end of the experiment indicate that protein supply exceeded needs in these groups. The larger the amount of BB in the diet, the higher the glucose level, according to our findings in the plasma metabolic profile (Table 4), and this is similar to results obtained by Monllor (2020). Due to the different behaviour throughout the experiment between treatments, there was a significant interaction of treatment with sampling in the two variables of glucose and urea. Glucose exhibited an elevation in the third treatment (T_3), however it was not significant. Blood rea significantly increased at sampling in the BB treatments. Metabolic Profile of the plasma glucose and urea levels were within the ranges deemed ideal for ewes despite changes in metabolic profiles between treatments (Kaneko *et al.* 1997; Desco *et al.* 1989; Dias 2010).

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