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# Response of Growing Ossimi Lambs to Diets Containing Different Levels of Defatted Jojoba Meal

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**Abstract:** Twenty growing Ossimi lambs were used to evaluate the effects of diets containing different levels of defatted Jojoba meal (JM) on performance, digestibility and blood metabolic profile tests. Replacement concentrate feed mixture by 0% (group 1), 10% (group 2), 20% (group 3) and 30% (group 4) JM (0, 33.3, 66.7 and 100%) in place of undecorticated cotton seed meal (CSM) caused a significant lower feed intake as dry matter, TDN, crude protein and digestible protein. Also, average daily gain (g/d) was significantly lower for the experimental groups 2 (97 g), 3 (61 g) and 4 (46 g) than the group 1 (216 g), which caused a significant higher feed conversion for the experimental groups 2,3 and 4. There were no significant differences in all digestion coefficients among the groups. Hemogram profile showed significant decreases in RBC's and PCV in lambs fed 30% JM and in the absolute numbers of eosinophils and lymphocytes in lambs fed 20 and 30% JM. Serum biochemical analysis showed higher significant increases in levels of total cholesterol, triglycerides, HDL-cholesterol and ALT in lambs fed 30% JM than those in the other groups. Both AST and GGT activities were significantly higher in the lambs fed 20 and 30% JM than those in the other groups. The endocrine function tests showed numerical non-significant increases in thyroid hormone levels ($T_3$ and $T_4$) in the three JM-treated groups, while the increase was significant for $T_4$ in the lambs fed 30% JM diet. Insulin levels decreased significantly in lambs fed 20 and 30% JM rations, while IGF-I showed significant decline in all levels of JM groups and reached a minimum at the end of trial for the groups of lambs fed 20 and 30% JM rations. No observable changes were detected in levels of glucose, LDL, creatinine and TSH.

**Key words:** Growing lambs · Defatted Jojoba meal · Performance · Digestibility · Blood metabolic profile tests

## INTRODUCTION

The scarcity and high price of protein ingredients for animal feeding highlight the need for close studies of all possible sources of protein for continued efficient animal production. Although traditional plant protein meal is the main protein source in major animal areas of the world, there is a need to consider alternatives. Several advantages are favoring Jojoba seed (JS) to be grown in Egypt such as a limited water requirement, high seed yield in new reclaimed soils and relatively high oil content 50% [1]. Jojoba meal (JM) represents a potential ingredient for animal feed, due to its high protein content 30% [1]. But the major deterrent to its use in animal feeding was the presence of several anti-nutritional factors, such as simmondsin and anti-trypsin factors [2-6].

The most important glycosidic component is simmondsin which reduces feed intake and body weight as well as its effect on the physiological functions [7-9]. Simmondsin may increase brown adipose tissue and metabolic rate by stimulating thyroid production which cause lower feed efficiency and decrease growth rate [8,10]. Other mechanisms involved in the fat loss properties of simmondsin include stimulation of thyroxin and growth hormone [11].

In addition, anemia was observed in rats fed 3% defatted JM [7]. At the microscopic level, Cokelaere *et al.* [12] detected that peripheral sinus of the mesenteric lymph nodes was filled with RBC's and there was active erythrophagocytosis in rats fed on feed with 0.25% purified simmondsin.

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There is a few published data on the response of sheep to diet containing JM. Consequently, these series of experiments were initiated to determine the relative acceptability of diets containing 10%, 20% and 30% untreated JM compare with diet containing concentrate feed mixture (30% CSM) on performance, digestibility and blood metabolic profile tests significant for hemogram, liver, kidney and endocrine functions of growing Ossimi lambs.

## MATERIALS AND METHODS

This study was carried out at Sheep and Goats Research Unit at El-Bostan area, Abd El-Monaiem Reyad Village, Nubaria El-Behaira government belonging to Animal Production Department and Parasitology and Animal Diseases Department, National Research Centre, Dokki, Cairo, Egypt to replace Jojoba meal (JM) in place of undecorticated cotton seed meal (0, 33.3, 66.7 and 100%) for rations 1, 2, 3 and 4, respectively.

**Feeds and Experimental Groups:** Twenty growing Ossimi lambs (average 20.80 kg) were divided into 4 similar groups according to live body weight (5 lambs in each group) and allotted randomly to 4 tested experimental rations. The growth trials lasted for 98 days. The experimental concentrate feed mixtures (CFM) are shown in Table 1 and 2. All rations were nearly isocaloric and isonitrogenous Table 3. Defatted JM was ground before mixing in the tested rations. All ingredients of each ration were mixed well. The experimental concentrate feed mixture and bean straw were offered *ad lib* in separate fodder to group feeding animals in a ratio of 70: 30 concentrate: roughage. Fresh water and mineral blocks were freely available at all times. The experimental lambs were individually weighed bi-weekly and offered feeds were weekly adjusted according to changes of body weight.

**Digestibility Trials:** At the end of the experiment, four digestibility trials were carried out using mature Ossimi rams aged 2.5-3 years old and 67 kg live body weight

Table 1: Chemical analysis of feed ingredients

| | Chemical composition on DM basis | | | | | |
|---|---|---|---|---|---|---|
| Ingredients | DM | CP | CF | EE | NFE | Ash |
| Yellow corn | 91.30 | 9.30 | 2.30 | 3.50 | 83.70 | 1.20 |
| Soybean meal | 90.78 | 44.21 | 6.67 | 2.27 | 40.12 | 6.73 |
| Undecorticated cotton seed meal | 87.88 | 21.82 | 27.75 | 2.71 | 38.92 | 5.80 |
| Defatted Jojoba meal | 94.30 | 28.24 | 11.00 | 12.20 | 44.12 | 4.44 |
| Wheat bran | 90.20 | 14.00 | 11.22 | 3.00 | 60.08 | 11.70 |
| Bean straw | 92.63 | 5.48 | 34.96 | 0.65 | 47.64 | 11.27 |

DM, dry matter; CP, crude protein; CF, crude fiber; EE, ether extract and NFE, nitrogen free extract

Table 2: Composition and chemical analysis of the concentrate feed mixtures (CFM)

| Ingredients | $CFM_1$ | $CFM_2$ | $CFM_3$ | $CFM_4$ |
|---|---|---|---|---|
| Yellow corn | 50.00 | 50.00 | 50.00 | 50.00 |
| Soybean meal | 6.80 | 6.80 | 6.80 | 6.80 |
| Undecorticated cotton seed meal | 30.00 | 20.00 | 10.00 | -- |
| Defatted Jojoba meal | -- | 10.00 | 20.00 | 30.00 |
| Wheat bran | 10.00 | 10.00 | 10.00 | 10.00 |
| Limestone | 2.00 | 2.00 | 2.00 | 2.00 |
| Sodium chloride | 1.00 | 1.00 | 1.00 | 1.00 |
| Vit. And Min. Mixture* | 0.20 | 0.20 | 0.20 | 0.20 |
| Chemical analysis on DM basis | | | | |
| DM | 90.24 | 90.89 | 91.53 | 92.17 |
| CP | 16.51 | 16.83 | 17.19 | 17.53 |
| CF | 11.05 | 9.37 | 7.70 | 6.02 |
| EE | 3.01 | 3.96 | 4.91 | 5.86 |
| NFE | 62.26 | 62.81 | 63.30 | 63.83 |
| Ash | 7.17 | 7.03 | 6.90 | 6.76 |

* Each 3 kg vitamins and minerals mixture contains: Vit. A 12,000,000 IU, Vit. $D_3$ 2,200,000 IU, Vit. E 10,000 mg, Vit. $K_3$ 2000 mg, Vit. $B_1$ 1000 mg, Vit. $B_2$ 5000 mg, Vit. $B_6$ 1500 mg, Vit. $B_{12}$ 10 mg, pantothenic acid 10,0 mg, Niacin 30,000 mg, Folic acid 1000 mg, Biotin 50 mg, Choline 300,000 mg, Manganese 60,000 mg, Zinc 50,000 mg, Copper 10,000 mg, Iron 30,000 mg, Iodine 100 mg, Selenium 100 mg, Cobalt 100 mg, $CaCo_3$ to 3,000 g.

Table 3: Composition and chemical analysis of the experimental rations

| Items | Ration (1) (0% JM) | Ration (2) (10% JM) | Ration (3) (20% JM) | Ration (4) (30% JM) |
|---|---|---|---|---|
| Composition of the experimental rations | | | | |
| Concentrate feed mixture % | 73.42 | 74.30 | 67.21 | 70.56 |
| Bean straw % | 26. 58 | 25.70 | 32.79 | 29.44 |
| Chemical analysis of the experimental rations on DM basis | | | | |
| DM | 90.87 | 91.34 | 91.89 | 92.31 |
| CP | 13.58 | 13.91 | 13.35 | 13.98 |
| CF | 17.40 | 15.94 | 16.64 | 14.54 |
| EE | 2.38 | 3.11 | 3.51 | 4.32 |
| NFE | 58.38 | 58.92 | 58.16 | 59.07 |
| Ash | 8.26 | 8.12 | 8.34 | 8.09 |
| GE (kcal / kg DM) * | 4146.00 | 4185.00 | 4189.00 | 4250.00 |

* GE (kcal / kg DM): Calculated according to Blaxter [47]. Each g CP = 5.65 kcal, g EE = 9.4 kcal and g (CF & NFE) = 4.15 kcal

(four animals in each group were used). Animals were housed in metabolic cages for ten days as a preliminary period followed by a seven days collection period. Feeds and feces were analyzed according to AOAC [13]. Drinking water was determined for each animal daily. A control container was also used to determine water losses due to evaporation. Total digestible nutrients (TDN) and digestible crude protein (DCP) of the different experimental rations were calculated as mentioned by Abou-Raya [14].

**Veterinary Care:** The animals proved to be free from internal and external parasites. All animals were kept during the period of experiment under close clinical observation, according to Kelly [15] and were not exposed to either stresses or pathogens.

**Blood Samples:** Two blood samples were individually collected via vein puncture, initially (zero-time) and on 45 and 90 days post treatments. The first was received into sterile ethylene-diamine tetra acetic acid (EDTA) coated vacutainer as anticoagulant for hematological studies. The second was collected without anticoagulant for separation of serum which was stored at -20°C for biochemical and hormonal analysis. Serum glucose was determined as soon as possible after sampling.

**Hematological Profile:** Total erythrocytic count (RBC's), packed cell volume (PCV), hemoglobin (Hb), total and differential leucocytic count (WBC's) and (DLC) were performed according to Jain [16]. Red Cell indices and absolute values of different white cells were calculated.

**Biochemical and Hormonal Assay:** Sera were analyzed for glucose, total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate amino transferase (AST), urea and creatinine using automated analyzer (Olympus chemistry analyzer AU 400, Olympus optical - Co. LTD), according to Young [17]. The activity of γ-glutamyl transferase (GGT) in serum was assayed spectrophotometrically using commercial kit, according to Persijn [18].

Sera were also analyzed for triiodothyronine ($T_3$), Thyroxin ($T_4$) and thyroid stimulating hormone (TSH) using Enzyme Immunoassay test kit (Monobind, INC, Costa Mesa, CA 92627, USA) and Eliza Reader Stat Fax-2100, according to Braveman [19]. Insulin and insulin like growth factor-I (IGF-I) were analyzed by radio immune-assay (RIA) kits for Diagnostic System Laboratories (DSL),Corporate Head Quarters 445 Medical Center-Webster, Texas 7-589-7217,USA. Insulin DSL-1600 and Insulin growth factor-1 (IGF-I) were determined according to Gerich [20] and Daughaday and Rofwein [21], respectively.

**Statistical Analysis:** Data collected were subjected to statistical analysis as one way analysis of variance according to Snedecor and Cochran [22]. Duncan's Multiple Range Test [23] was used to separate means when the dietary treatment effect was significant. On the other hand blood plasma data were subjected to statistical analysis as two factorial analysis of variance

## RESULTS AND DISCUSSION

The proximate analysis of feed ingredients and defatted JM are shown in Table 1. The present results are in general agreement with those reported by Motawe [5]; Sobhy *et al.* [9]; Verbiscar and Benigan [24]; Verbiscar *et al.* [25] and Ngoupayou *et al.* [26]. . The moisture content of defatted JM was less than 6% indicating the possibility of storing such materials for a long time

without deleterious effect. The lower level of crude fiber (CF) in JM would increase its nutritive value for ruminants.

It was noticed that the high crude protein (CP) content (28.24%) in JM was associated with a lower CF content (11.00%). This would be an indication of the superiority of JM in its nutritive value for ruminant feeding. A comparison was made to evaluate the tested material with soybean meal (SBM), rapeseed meal (RSM) and cotton seed meal (CSM) for CP content (%). The results showed that 28.24% CP for JM compared with 42.6, 35.0 and 47.5% CP for SBM, RSM and CSM, respectively. However, the CP content of JM lower than its content in other feedstuffs in the comparison.

Ether extract (EE) of JM (12.20%) was markedly higher than most of published values [25, 26 and 27] and lower than that obtained by Sobhy *et al.* [9]. It is well know that the solvent extraction method is more efficient in terms of oil removal and less than 2% of the oil could remain in the meal. So press extraction meals on the other hand, usually contain higher residual oil. The variation in EE among JM could be attributed to the differences among the steps of methods of extraction. However, the wide variation in the chemical composition of JM among the reported values in the literature and those of the tested sample could be mainly attributed to the different varieties of cultivars used. Moreover, the values may widely depending on the degree of processing method oil extraction procedure and even the size of seed [28].

The composition of different experimental rations are shown in Table 3. All rations were isonitrogenous and isoenergetic. Proportional replacement (w/w) undecorticated CSM by defatted JM (33.3%, 66.7% and 100%) did not effectively influenced the chemical analysis of the feed mixtures except CF content. The difference between the experimental groups in CF content attribute to replacement CSM with JM along with bean straw percent.

Feed intake values expressed as CFM or in terms of dry matter (g/d) and TDN (g/d) in Table 4 were significantly lower for the experimental groups (2, 3 and 4) than group 1. Only the differences between groups 1 and 4 (TDN) were not significant. However, no significant differences were observed between the experimental groups 2,3 and 4. These data confirm with the observation of Trei *et al.* [29] with sheep and extend them to cattle, i.e. untreated JM has a depressing effect on feed intake by ruminants, just as it dose in non-ruminants. Lambs appeared to be more sensitive to, or at least more responsive to the appetite depressing factors in JM than steers, suggesting that results of JM experiments obtained with one of the species may not apply directly to the other.

Moreover, Swingle *et al.* [30] found that steers fed the diet with 10% untreated JM for over 60 days showed no detrimental effects other than those that could be attributed to reduce feed intake. When the JM diet was discontinued, feed consumption and animal condition

Table 4: Dry matter, energy and nutrient intakes by the experimental group lambs

| | Experimental group lambs | | | | |
|---|---|---|---|---|---|
| Item | Group (1) | Group (2) | Group (3) | Group (4) | SEM |
| Av. Body weight, kg* | 31.60$^{a}$ | 25.65$^{b}$ | 23.70$^{b}$ | 23.05$^{b}$ | 1.08 |
| Metabolic body size** | 13.23$^{a}$ | 11.40$^{b}$ | 10.74$^{b}$ | 10.52$^{b}$ | 0.36 |
| Feed intake as: | | | | | |
| CFM (g/day) | 1137.00$^{a}$ | 820.00$^{b}$ | 632.00$^{b}$ | 722.00$^{b}$ | 42.70 |
| Bean straw (g/day) | 146.00$^{b}$ | 125.00$^{b}$ | 162.00$^{b}$ | 249.00$^{a}$ | 11.90 |
| Dry matter: | | | | | |
| g / day | 1283.00$^{a}$ | 945.00$^{b}$ | 794.00$^{b}$ | 971.00$^{b}$ | 45.60 |
| g / Kg W$^{0.75}$ | 97.02$^{a}$ | 82.89$^{ab}$ | 73.93$^{b}$ | 92.30$^{ab}$ | 3.60 |
| TDN: | | | | | |
| g / day | 804.00$^{a}$ | 632.00$^{b}$ | 551.00$^{b}$ | 692.00$^{ab}$ | 28.90 |
| g / Kg W$^{0.75}$ | 60.77$^{ab}$ | 55.44$^{ab}$ | 51.30$^{b}$ | 65.78$^{a}$ | 2.40 |
| Crude protein: | | | | | |
| g / day | 174.00$^{a}$ | 132.00$^{b}$ | 106.00$^{b}$ | 136.00$^{b}$ | 6.30 |
| g / Kg W$^{0.75}$ | 13.15$^{a}$ | 11.58$^{ab}$ | 9.87$^{b}$ | 12.93$^{a}$ | 0.50 |
| Digestible crude protein: | | | | | |
| g / day | 100.00$^{a}$ | 78.00$^{bc}$ | 63.00$^{c}$ | 84.00$^{ab}$ | 3.70 |
| g / Kg W$^{0.75}$ | 7.56$^{a}$ | 6.84$^{ab}$ | 5.86$^{b}$ | 7.98$^{a}$ | 0.30 |

*Av. Body weight, Kg = Initial weight - Final weight/2.

** metabolic body size = Kg W$^{0.75}$

a,b and c: Means in the same row having different superscripts differ significantly at level ($P<0.05$).

Table 5: Growth performance of the experimental group lambs

| | Experimental group lambs | | | | |
|---|---|---|---|---|---|
| Item | Group (1) | Group (2) | Group (3) | Group (4) | SEM |
| No. of animals | 5.00 | 5.00 | 5.00 | 5.00 | --- |
| Initial weight, Kg | 20.70 | 20.90 | 20.70 | 20.80 | 0.70 |
| Final weight, Kg | 41.90$^{a}$ | 30.40$^{b}$ | 26.70$^{b}$ | 25.30$^{b}$ | 1.77 |
| Gain, Kg | 21.20$^{a}$ | 9.50$^{b}$ | 6.00$^{bc}$ | 4.50$^{c}$ | 1.60 |
| Experimental duration, days | 98.00 | 98.00 | 98.00 | 98.00 | --- |
| ADG, g/day | 216.00$^{a}$ | 97.00$^{b}$ | 61.00$^{c}$ | 46.00$^{c}$ | 16.30 |
| Relative gain (% of initial weight)* | 102.00$^{a}$ | 45.00$^{b}$ | 29.00$^{b}$ | 22.00$^{b}$ | 8.45 |
| Feed intake, g as: | | | | | |
| Dry matter | 1283.00$^{a}$ | 945.00$^{b}$ | 794.00$^{b}$ | 971.00$^{b}$ | 45.60 |
| TDN | 804.00$^{a}$ | 632.00$^{b}$ | 551.00$^{b}$ | 692.00$^{ab}$ | 28.90 |
| CP | 174.00$^{a}$ | 132.00$^{b}$ | 106.00$^{b}$ | 136.00$^{a}$ | 6.30 |
| DCP | 100.00$^{a}$ | 78.00$^{bc}$ | 63.00$^{c}$ | 84.00$^{ab}$ | 3.70 |
| Feed conversion (Kg. intake/Kg. gain) of | | | | | |
| Dry matter | 5.94$^{b}$ | 9.74$^{b}$ | 13.02$^{ab}$ | 21.11$^{a}$ | 2.17 |
| TDN | 3.72$^{b}$ | 6.52$^{b}$ | 9.03$^{b}$ | 15.04$^{a}$ | 1.57 |
| CP | 0.81$^{b}$ | 1.36$^{b}$ | 1.74$^{b}$ | 2.96$^{a}$ | 0.30 |
| DCP | 0.46$^{b}$ | 0.80$^{b}$ | 1.03$^{b}$ | 1.83$^{a}$ | 0.19 |

*Relative gain (% of initial weight)= Gain / initial weight x 100

a, b and c: Means in the same row having different superscripts differ significantly at level ($P<0.05$).

ADG, average daily gain; TDN, total digestible nutrients; CP, crude protein and DCP, digestible crude protein

improved accordingly. Also, Cokelaere *et al.* [31] concluded that simmondsin induced feed intake reduction by stimulating satiation. Furthermore, Trei *et al.* [29] reported that simmondsin was metabolized by rumen microorganisms *in vitro* and that only trace amount of the major toxicants could be detected in feces of lambs fed diets containing 10% JM.

The values of CP intake were significant lower for experimental groups 2, 3 and 4 than group 1. Concerning the values of DCP intake group 3 recorded the lower value than the other groups, the difference between group 4 and group 1 were not significant.

Booth *et al.* [3] and Verbiscar *et al.* [32] showed that simmondsin is broken its aglycon in the intestinal tract by the intestinal bacteria and that the aglycon (or a derivative of it) is responsible for the feed intake reduction. This aglycon is less hydrophilic and could possibly cross the intestinal wall more easily.

Average body weight gain, feed intake and feed conversion are given in Table 5. Average daily gains (ADG) were significantly lower for groups 2, 3 and 4 that fed 10, 20 and 30% JM compared to the group 1 (control). However, no significant difference was detected between groups 3 and 4. These data showed that inclusion of JM at different levels failed to support normal growth of sheep. Similar results obtained by Ngoupayou *et al.* [33] when deoiled untreated JM was included in rabbit diets at levels 5, 10 or 15% diet caused no mortality to weaning rabbits but failed to support normal growth. Jojoba oil meal had about 1.5% tannic acid but diets with up 3% tannic acid had no adverse effect on performance of rabbits. Also, Manos *et al.* [27] noticed that weight gains were significantly lower for the ewes fed the 10% JM compared to the control ($P<0.01$) or 5% JM ($P<0.05$) rations. The ewes fed the 5% JM showed a weight gain numerically lower than the corresponding control, but not significant. The wethers fed 5 or 10% JM showed numerically lower weight gain than the control but the differences were not significant.

Feed conversion values were differ among groups but the best values were recorded with group 1 (control). There were no significant difference between groups (1, 2 and 3), only, the differences between group 4 and other groups were significant.

The groups fed 10, 20 or 30% JM showed numerically higher digestion values (DM, OM, CP, CF, EE and NFE) than the group 1 (control), but the differences were not significant and consequently did not significant improvement in nutritive values in terms of TDN and DCP (Table 6). However, Swingle *et al.* [30] found that the digestibility of nitrogen was 10% lower ($P<0.05$) in the treated JM with lactobacillus acidophilus diet than in the CSM diet. Digestibilities of dry matter, organic matter and gross energy were also lower ($P<0.05$) in the treated JM

Table 6: Nutrient digestibilites and nutritive values of the experimental rations

| Item | Experimental group lambs: Ration (1) (0% JM) | Ration (2) (10% JM) | Ration (3) (20% JM) | Ration (4) (30% JM) | SEM |
|---|---|---|---|---|---|
| Nutrient digestibilities | | | | | |
| DM | 78.20 | 72.69 | 85.81 | 84.42 | 2.80 |
| OM | 66.38 | 69.93 | 72.44 | 73.26 | 1.73 |
| CP | 57.65 | 59.35 | 59.41 | 61.79 | 2.06 |
| CF | 36.72 | 47.49 | 51.84 | 49.68 | 3.13 |
| EE | 60.49 | 68.25 | 67.76 | 72.50 | 2.26 |
| NFE | 77.49 | 78.59 | 81.60 | 81.84 | 1.34 |
| Nutritive values | | | | | |
| TDN | 62.70 | 66.90 | 69.38 | 71.26 | 1.75 |
| DCP | 7.82 | 8.25 | 7.93 | 8.64 | 0.29 |

DM, dry matter; OM, organic matter; CP, crude protein; CF, crude fiber; EE, ether extract; NFE, nitrogen free extract; TDN, total digestible nutrients and DCP, digestible crude protein.

Table 7: Water metabolism as affected by the experimental rations

| Item | Experimental group lambs: Ration (1) (0% JM) | Ration (2) (10% JM) | Ration (3) (20% JM) | Ration (4) (30% JM) | SEM |
|---|---|---|---|---|---|
| Water intake, ml/h/d | | | | | |
| Drinking water | 3693 | 3956 | 3821 | 4113 | 188 |
| Feed water | 167[a] | 153[a] | 124[b] | 127[b] | 6 |
| Total | 3860 | 4109 | 3945 | 4240 | 188 |
| Water loss, ml/h/d | | | | | |
| Urinary loss | 1641[b] | 1585[b] | 1608[b] | 2258[a] | 111 |
| Fecal loss | 611 | 512 | 537 | 379 | 43 |
| Total water | 2252 | 2097 | 2145 | 2637 | 98 |
| Insensible loss, ml/h/d | 1608 | 2012 | 1800 | 1603 | 140 |

a and b: Means in the same row having different superscripts differ significantly at level ($P<0.05$)

diet, but these differences could largely be accounted for by the depression in protein digestibility. These data are in contrary with our results and that may be due to inadequate adaptation period during the digestibility trials.

Data of water metabolism expressed as (ml/h/d) of the experimental groups are given in Table 7. Drinking water values showed slightly non significant increase with JM diets than the group 1 (control) and that may be due to animals tend to consume more water for get rid of antinutritional factor in JM as simmondsin content. The reduced water intake in JM diets was entirely due to the feed intake reduction. These observations are in favor of the hypothesis that JM induces its feed intake reduction by stimulating satiety [31]. Insensible water loss (ml/h/d) values were not significantly differ between the experimental groups.

Clinical examination of the experimental animals showed marked emaciation in the group of lambs fed 30% JM-treated ration with no clinical signs of toxicity, anemia or hypothyroidism.

Table 8 lists the values of sheep metabolic profile tests that include hemogram, serum biochemical and hormonal analysis among the different JM dietary treatments, during the three periods of the experiment.

Concerning the hematological parameters, RBC's and PCV were significantly ($P<0.05$) lower in lambs fed 30% JM than in the corresponding controls, during the periods 2 and 3 of the experiment. There were no indications for severe anemia as shown in the red cell indices (MCV, MCH and MCHC),with no reticulocytes have been observed. Compared to control, all JM-treated groups, showed a non-significant ($P>0.05$) lower total number of WBC's in the second and third periods, while there were

Table 8: Effects of feeding experimental rations on blood metabolic profile tests of the experimental groups

| | Period No.(1) (Zero-time) | | | | Period No.(2)(After 1.5 month) | | | | Period No.(3)(After 3 months) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | 0% JM | 10% JM | 20% JM | 30% JM | 0% JM | 10% JM | 20% JM | 30% JM | 0% JM | 10% JM | 20% JM | 30% JM | SEM |
| 1-Hematological profile | | | | | | | | | | | | | |
| RBCs (x $10^6$µl) | 10.17$^{a}$ | 9.83$^{ab}$ | 9.41$^{ab}$ | 10.00$^{a}$ | 10.17$^{a}$ | 9.35$^{ab}$ | 9.10$^{ab}$ | 8.17$^{b}$ | 10.33$^{a}$ | 9.30$^{ab}$ | 8.87$^{ab}$ | 8.29$^{b}$ | 0.18 |
| Hemoglobin (g/dl) | 8.00$^{b}$ | 11.50$^{a}$ | 10.83$^{ab}$ | 11.00$^{a}$ | 11.33$^{a}$ | 11.50$^{a}$ | 10.57$^{ab}$ | 10.67$^{ab}$ | 11.50$^{a}$ | 11.33$^{a}$ | 10.17$^{ab}$ | 10.50$^{ab}$ | 0.29 |
| Hematocrit (PCV%) | 30.67$^{ab}$ | 31.00$^{ab}$ | 31.67$^{a}$ | 30.33$^{ab}$ | 30.33$^{ab}$ | 29.33$^{abc}$ | 29.67$^{abc}$ | 27.00$^{c}$ | 31.00$^{ab}$ | 28.67$^{ab}$ | 28.33$^{bc}$ | 27.00$^{c}$ | 0.35 |
| MCV (fl) | 30.37 | 31.80 | 34.18 | 31.44 | 30.17 | 31.52 | 32.68 | 33.25 | 30.04 | 31.03 | 32.05 | 33.10 | 0.54 |
| MCH(pg) | 11.18$^{ab}$ | 11.78$^{ab}$ | 11.62$^{ab}$ | 11.01$^{b}$ | 11.33$^{ab}$ | 12.29$^{ab}$ | 11.73$^{ab}$ | 13.16$^{a}$ | 11.15$^{ab}$ | 12.21$^{ab}$ | 11.49$^{ab}$ | 12.82$^{ab}$ | 0.21 |
| MCHC (g/dl) | 37.08 | 37.58 | 34.31 | 36.63 | 37.39 | 39.12 | 36.07 | 39.53 | 37.53 | 38.61 | 34.72 | 37.92 | 0.58 |
| WBCs (x $10^3$µl) | 8.52 | 9.67 | 9.08 | 9.05 | 9.05 | 8.38 | 8.28 | 8.77 | 9.02 | 8.70 | 7.80 | 8.33 | 0.19 |
| DLC(Absolute values, x $10^3$µ1) | | | | | | | | | | | | | |
| Neutrophils | 3.85 | 3.96 | 3.94 | 4.08 | 3.89 | 4.07 | 4.00 | 4.56 | 4.12 | 4.88 | 4.59 | 4.93 | 0.14 |
| Eosinophils | 0.41$^{abc}$ | 0.49$^{ab}$ | 0.39$^{abcd}$ | 0.39$^{abcd}$ | 0.50$^{a}$ | 0.24$^{cdef}$ | 0.24$^{cdef}$ | 0.17$^{ef}$ | 0.30$^{bcde}$ | 0.20$^{def}$ | 0.10$^{f}$ | 0.11$^{ef}$ | 0.03 |
| Lymphocytes | 4.03$^{bcd}$ | 4.96$^{a}$ | 4.46$^{ab}$ | 4.35$^{abc}$ | 4.35$^{abc}$ | 3.66$^{bcde}$ | 3.28$^{de}$ | 3.57$^{cde}$ | 4.41$^{abc}$ | 3.12$^{e}$ | 2.87$^{e}$ | 2.94$^{e}$ | 0.13 |
| Monocytes | 0.23$^{bc}$ | 0.26$^{abc}$ | 0.30$^{abc}$ | 0.24$^{bc}$ | 0.32$^{abc}$ | 0.42$^{abc}$ | 0.28$^{abc}$ | 0.47$^{a}$ | 0.20$^{c}$ | 0.44$^{ab}$ | 0.24$^{bc}$ | 0.35$^{abc}$ | 0.02 |
| 2-Biochemical analysis | | | | | | | | | | | | | |
| Glucose (mg/dl) | 56.00 | 58.00 | 53.69 | 52.80 | 52.83 | 53.96 | 62.33 | 60.00 | 53.07 | 63.10 | 62.33 | 64.67 | 1.37 |
| Total cholesterol(mg/dl) | 79.67$^{c}$ | 86.17$^{c}$ | 82.63$^{c}$ | 82.83$^{c}$ | 82.33$^{c}$ | 80.33$^{c}$ | 91.00$^{bc}$ | 106.67$^{ab}$ | 83.23$^{c}$ | 82.83$^{c}$ | 89.83$^{c}$ | 116.33$^{a}$ | 2.69 |
| Triglycerides (mg/dl) | 47.00$^{e}$ | 51.83$^{bcde}$ | 54.17$^{abcd}$ | 47.67$^{e}$ | 48.83$^{de}$ | 50.67$^{cde}$ | 50.33$^{cde}$ | 56.67$^{abc}$ | 51.67$^{bcde}$ | 47.50$^{e}$ | 49.83$^{de}$ | 59.67$^{a}$ | 0.82 |
| HDL-cholesterol(mg/dl) | 27.67$^{cd}$ | 25.70$^{cd}$ | 24.17$^{cd}$ | 26.43$^{cd}$ | 24.83$^{cd}$ | 22.04$^{d}$ | 30.27$^{c}$ | 31.67$^{bc}$ | 26.50$^{cd}$ | 29.50$^{cd}$ | 41.33$^{a}$ | 39.67$^{ab}$ | 1.17 |
| LDL-cholesterol (mg/dl) | 47.50 | 45.17 | 47.33 | 39.43 | 48.33 | 43.67 | 45.67 | 44.00 | 47.83 | 44.33 | 49.33 | 47.00 | 1.19 |
| ALT (U/L) | 13.43$^{bc}$ | 12.24$^{c}$ | 15.00$^{bc}$ | 14.77$^{bc}$ | 13.84$^{bc}$ | 16.24$^{bc}$ | 15.87$^{bc}$ | 19.00$^{ab}$ | 14.45$^{bc}$ | 13.95$^{bc}$ | 17.50$^{abc}$ | 23.00$^{a}$ | 0.66 |
| AST (U/L) | 58.54$^{b}$ | 70.00$^{b}$ | 56.97$^{b}$ | 56.37$^{b}$ | 49.80$^{b}$ | 57.07$^{b}$ | 105.00$^{a}$ | 109.33$^{a}$ | 55.23$^{b}$ | 66.00$^{b}$ | 116.67$^{a}$ | 125.33$^{a}$ | 4.97 |
| GGT (U/L) | 43.63$^{b}$ | 36.67$^{b}$ | 39.93$^{b}$ | 41.50$^{b}$ | 41.73$^{b}$ | 38.43$^{b}$ | 69.10$^{a}$ | 68.93$^{a}$ | 39.75$^{b}$ | 46.10$^{b}$ | 69.00$^{a}$ | 73.22$^{a}$ | 2.66 |
| Urea (mg /dl) | 32.50 | 29.54 | 30.33 | 30.33 | 30.50 | 28.53 | 33.00 | 31.67 | 31.00 | 36.00 | 33.67 | 34.67 | 1.07 |
| Creatinine (mg/ dl) | 0.84 | 0.76 | 0.85 | 0.87 | 0.87 | 0.77 | 0.83 | 0.86 | 0.87 | 0.83 | 0.93 | 0.80 | 0.03 |
| 3-Hormonal analysis | | | | | | | | | | | | | |
| $T_3$ (ng/ml) | 136.00$^{ab}$ | 1.39$^{ab}$ | 1.67$^{ab}$ | 1.39$^{ab}$ | 1.20$^{b}$ | 1.47$^{ab}$ | 1.33$^{b}$ | 2.05$^{ab}$ | 1.41$^{ab}$ | 1.41$^{ab}$ | 2.34$^{a}$ | 2.01$^{ab}$ | 0.09 |
| $T_4$ (µg/ml) | 10.73$^{bc}$ | 11.15$^{bc}$ | 9.97$^{c}$ | 10.08$^{bc}$ | 10.04$^{bc}$ | 10.00$^{bc}$ | 12.77$^{ab}$ | 11.13$^{bc}$ | 9.92$^{c}$ | 10.61$^{bc}$ | 10.20$^{bc}$ | 14.33$^{a}$ | 0.32 |
| TSH (µIU/ml) | 2.10$^{abc}$ | 1.54$^{bc}$ | 1.44$^{c}$ | 1.37$^{c}$ | 1.63$^{abc}$ | 1.76$^{abc}$ | 2.34$^{a}$ | 2.28$^{ab}$ | 2.05$^{abc}$ | 1.97$^{abc}$ | 1.90$^{abc}$ | 2.10$^{abc}$ | 0.08 |
| Insulin (µIU/ml) | 5.43$^{abc}$ | 6.20$^{a}$ | 5.59$^{abc}$ | 5.96$^{ab}$ | 6.27$^{a}$ | 6.27$^{a}$ | 4.77$^{c}$ | 5.00$^{bc}$ | 5.92$^{ab}$ | 6.03$^{a}$ | 4.87$^{c}$ | 4.64$^{c}$ | 0.13 |
| IGF-I (ng/ml) | 137.43$^{abc}$ | 118.00$^{cdef}$ | 137.83$^{abcd}$ | 155.10$^{a}$ | 145.67$^{ab}$ | 110.13$^{defg}$ | 95.00$^{fg}$ | 91.50$^{g}$ | 134.33$^{abcd}$ | 121.00$^{bcde}$ | 105.10$^{efg}$ | 88.60$^{g}$ | 4.12 |

a, b, c, d, e, f and g : Means in the same row having different superscripts differ significantly at level (P<0.05)

significant (P<0.05) decreases in the absolute values of eosinphils and lymphocytes in lambs fed 20 and 30% JM diets during the last two periods of the experiment. Similar results were obtained by Manos *et al.* [27] in sheep and Cokelaere *et al.* [7] and Sobhy *et al.* [9] in rats, who reported non-significant decrease in hematological parameters in animals fed 3, 5 and 10% JM rations. Taking into account the relative protein shortage induced by JM, it is argued that the slight changes in the hemogram could be provoked by this phenomenon [7,34], although the increased erythrophagocytosis in the mesenteric lymph nodes could be involved as well [12]. Further investigations are needed to elucidate this problem.

Serum biochemical analysis (Table 8) included glucose, lipids (cholesterol and triglycerides), lipoproteins (HDL-cholesterol and LDL-cholesterol), enzymes (ALT, AST and GGT), urea and creatinine. Total cholesterol and triglycerides levels were significantly higher (P<0.05) in lambs fed 30% JM ration than in those fed 0 and 10% ones during the second period of the experiment, while these parameters were higher significantly (P<0.05) than in 0, 10 and 20% JM diets at the end of experiment. The HDL concentration was significantly higher (P<0.05) in lambs fed the 30% JM diet compared with those of 0 and 10% groups, during the second and third periods of experiment. The obtained results agreed with Flo *et al.*[8]

and Sobhy *et al.* [9] in rats. The increase in total cholesterol levels could be explained by the increase in HDL-cholesterol. The significant increase in lipid profile in lambs fed the high concentration of JM in ration (30%), indicated that there was mobilization of fatty acids from fat depot which may be due to release of cortisol in response to the stress of nutrition [35].

In the present study, the activity of ALT enzyme was significantly higher ($P<0.05$) in lambs fed 30% JM ration than those fed 0 and 10%, but not 20% at the end of the experiment. However, both AST and GGT were significantly higher ($P<0.05$) in lambs fed the 20 and 30% JM rations than the other two-dietary treatment groups, during the second and third periods of experiment. Manos *et al.*[27] recorded a significantly increased GGT in ewes fed 5 and 10% JM rations, while Sobhy *et al.* [9] observed significantly elevation in AST and ALT levels in rats fed 3 and 6% JM. The obtained enzymatic profile, indicated problems in liver or damage to a variety of tissues in lambs fed 20 and 30% JM rations, which increased blood levels of ALT, AST and GGT.

As shown in Table 8, there were noticeable but non-significant increase in values of urea in lambs fed the JM rations as compared with the corresponding controls. There are contradictory findings regarding the effect of JM on blood urea nitrogen. Manos *et al.* [27]recorded a significant decrease in blood urea-nitrogen in lambs fed 10% JM ration, while Sobhy *et al.* [9], reported a significant increase in the same parameter in rats fed 3 and 6% JM diets. The increase of urea could be explained by a relative protein shortage-due to fed on JM rations-which results in breakdown of body tissues to compensate for animal's nutritional needs. The breakdown of the body proteins provides this, but at the expense of muscle mass and the release of nitrogenous compounds which increase blood urea nitrogen [36]. No observable changes were detected in the concentrations of glucose, LDL-cholesterol and creatinine in any of the JM-treated groups.

The endocrine function tests included analysis of thyroid hormones ($T_3$,$T_4$ and TSH), insulin and IGF-I in attempt to elucidate the underlying casual mechanisms of the observed emaciation in lambs fed the high concentration of JM. As shown in Table 8, serum $T_3$ and $T_4$ levels showed numerical non-significant ($P>0.05$) increase during the second and third periods of experiment in the three JM dietary treatment groups, otherwise the increase was significant ($P<0.05$) for $T_4$ in the group fed 30% JM at the end of experiment as compared with the other groups. No observable differences were detected between control and JM-treated groups for TSH. Supplementation of 20 and 30% JM rations significantly ($P<0.05$) decreased serum insulin levels as compared with control and 10% JM groups, during the second and third periods of experiment. These results coincide with those reported previously by Arnouts *et al.* [11] in poultry and by Cokelaere *et al.* [7] and Flo *et al.* [8 and 10] in rats. The same authors recorded that simmondsin reduces the body weight due to its effect on thyroid hormones and insulin. The $T_3$ and $T_4$ increases could be explained by a relative protein shortage induced by simmondsin [37 and 38]. The higher $T_3$ and $T_4$ concentrations are indicative of high heat production [39 and 40]. The higher energy dissipation causes decreased food efficiency [37] which explains the emaciating effect of JM. The serum cholesterol levels generally vary inversely with thyroid activity [41 and 42]. On the contrary the obtained results showed no correlation between the concentrations of thyroid hormones and cholesterol levels, as previously demonstrated by Nazifi *et al.* [43 and 44] in sheep and goats.

The major role of IGF-I is the regulation of tissue growth and differentiation [45]. The authors suggested that this hormone is regulated by nutrition. Serum IGF-I levels showed gradual significant decrease ($P<0.05$) in the three JM-treated rations and reached a minimum at the end of experiment for the groups of 20 and 30% JM supplementation. Similar records were given by Huybrechts *et al.* [46] and Arnouts *et al.* [11] in poultry. The authors suggested that JM gave effect similar with feed restriction to the point of decreasing IGF-I secretion. The obtained results of the organ function tests in the present study disagreed with that previously obtained by Trei *et al.* [29], who reported that lambs fed diets containing up to 30% untreated JM had no discernible changes in blood constituents.

## CONCLUSION

Because of the marked detrimental effect of defatted JM on feed intake, gain, physiological and endocrinological functions, it seems unlikely that untreated JM could become an important feed ingredient for productive livestock. Future studies should be needed to evaluate the antinutritional factor in JM and try to reduce or remove the content of simmondsin by different methods such ensiling and biological treatments (second paper).

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