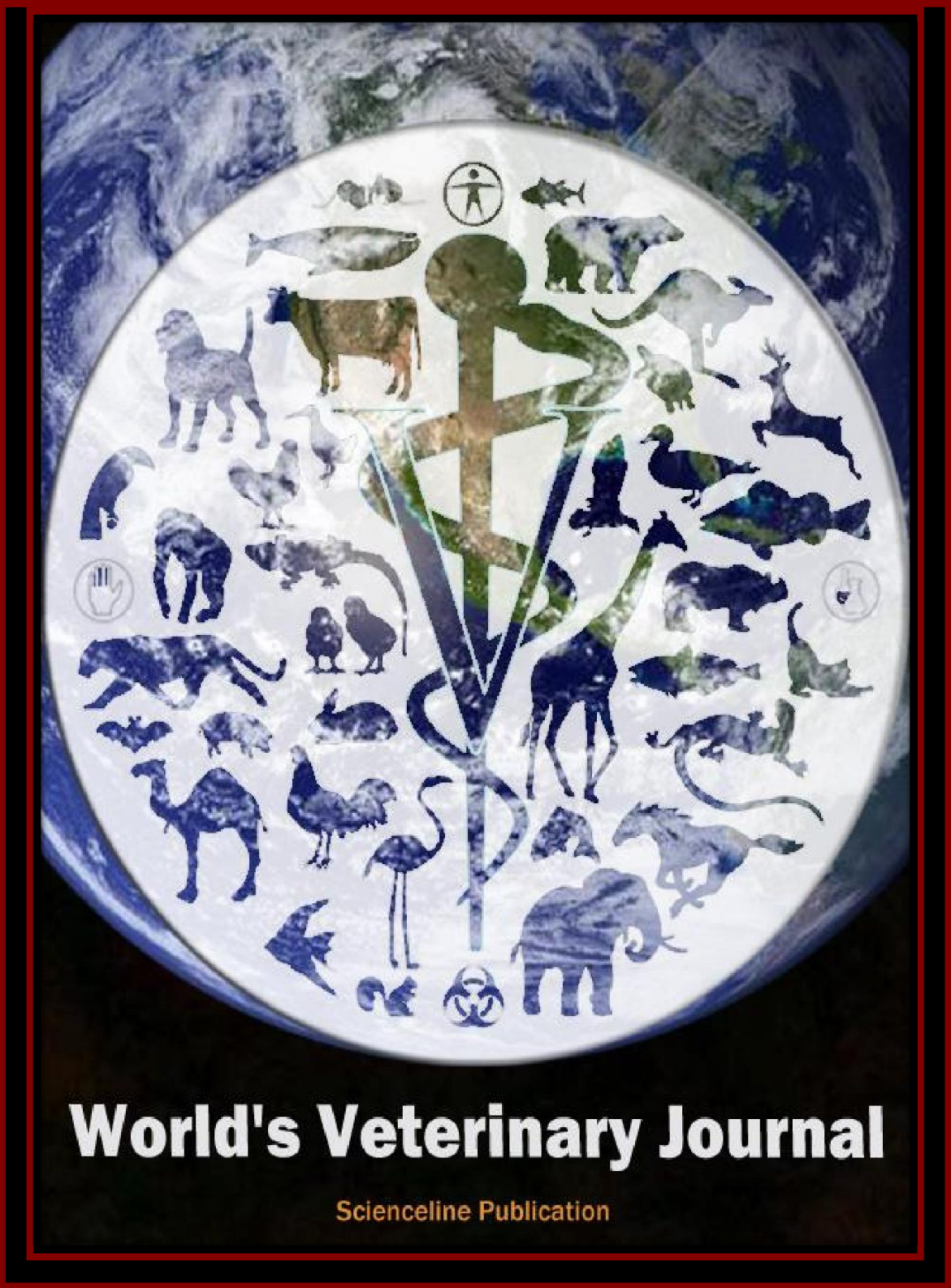

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World Vet. J. 9(3): 174-180, 2019; pii:S232245681900022-9
DOI: https://dx.doi.org/10.36380/scil.2019.wvj22

ABSTRACT
Ruminant meat quality is one of the important factors contributing to the recent spreading of several diseases, such as obesity, cancer, and cardiovascular problems, which have increased predominately. Feeding regiment plays an important role in the determination of the composition of fatty acids and meat quality in ruminants. This review aims to highlight the main factors that lie behind the variability of ruminant meat quality and its effect on human being's health. The reduction in grass-feeding decreases saliva levels in the ruminants, which has several consequences on the rumen, including a reduction in pH level, along with a reduction in the microorganism activities and conjugated linoleic acid levels. In adipose tissues, the expression of the stearoyl-CoA desaturases gene is negatively affected by the decreased conjugated linoleic acid levels in the rumen, which leads to a decreased transformation of saturated fatty acids to monounsaturated fatty acids. Therefore, the lower monounsaturated fatty acids and the parallel increase in the proportion of saturated fatty acids in the consumed meat can be associated with some human diseases. Thus, the present study provided a molecular explanation for the superiority of grass-based feeding in ruminants raised at pasture in term of production of meat with a healthier quality for consumers than those raised on grains.

Key words: Grain; Grass, Human disease, Ruminant meat, SCD enzyme

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Research Paper

Characterization of Pregnancy-Associated Glycoprotein as a Biomarker of Pregnancy in Etawa Crossbred Goat.

Ningtyas IK, Lestari TD and Hermadi HA.
World Vet. J. 9(3): 181-186, 2019; pii:S232245681900023-9
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ABSTRACT
Pregnancy-Associated Glycoprotein (PAG) is secreted by the placenta, which is produced in mononucleate and binucleate trophoblast cells. The current research was conducted to find out a substance for diagnosing early pregnancy in Etawa crossbred goats. Six Etawa crossbred goats (not pregnant, three months pregnant and four months pregnant) were subjected in the present study from Livestock Government Institution Breeding in Singosari, Malang. The research methods consisted of sample collection, identification PAG with sodium dodecyl sulfate–polyacrylamide gel electrophoresis, the determination of concentration with Biuret method and specificity test with Western Blot assay. The obtained results showed that the molecular weight of PAG from pregnant Etawa crossbred goats was 55.85 kDa. The average concentrations of PAG in the goats of non-pregnant, three months pregnant, and four months pregnant were 1.83±2.98, 3.79±2.72 and 4.36±2.63, respectively. The results of the specificity test with the Western Blot molecular revealed a molecular mass of PAG was 55 kDa. The findings of the present study demonstrated PAG in Etawa crossbred goats can be used as an indicator of pregnancy.

Key words: Biomarkers, Etawa crossbred, PAG, Pregnancy

Research Paper

Incretin Mimetics Vildagliptin and Exenatide Improve Pedicle Skin Flap Survival in Rats.

Danilenko LM, Tarasova AP, Pokrovskiy MV, Trunov KS, Stepenko YV, Artyushkova EB and Gudyrev OS.
World Vet. J. 9(3): 187-191, 2019; pii:S232245681900024-9
DOI: https://dx.doi.org/10.36380/scil.2019.wvj24

ABSTRACT
Hypoxia and tissue ischemia are the leading factors in the alteration of tissues in many pathological conditions. Prevention and reversion of the effects of local ischemia, which develops during various surgical interventions, is an actual problem of modern medicine. The aim of the present study was to investigate the effect of exenatide and vildagliptin on the survival rate of an isolated pedicle skin flap in sixty adults Wistar rats. Simulation of a pedicle skin graft was performed on the second day of the experiment. After anesthesia under aseptic conditions, a skin graft was cut out: isolated in a plastic bag, the edges of the skin were stitched with interrupted sutures (nylon 3/0). Rats were divided into six groups: control group, exenatide group (10 µg/kg/day subcutaneously for nine days after surgery), vildagliptin group (0.2 mg/kg/day intraperitoneally for nine days after surgery) and pentoxifylline group (100 mg/kg/day intravenously, two hours before the surgical intervention). In the other two groups, glibenclamide (5 mg/kg) were administered before injection of incretin mimetics. On the third, seventh and tenth day, area of the surviving tissue was measured. Subsequently, the survival rate of the skin graft was calculated. The area of the surviving tissue in exenatide and vildagliptin group was 1.5 and 1.7 times more compared to the control group, respectively. Preliminary blockade of ATP-dependent potassium channels by glibenclamide eliminated the protective effect of exenatide and vildagliptin. The increase in the survival of ischemic tissues using exenatide and vildagliptin has been experimentally proved. The current study confirmed the important role of ATP-dependent potassium channels in dermatoprotective properties of incretin mimetics.

Key words: Dermatoprotective properties, Exenatide, Ischemia, Pedicle skin graft, Vildagliptin.

[Full text-PDF][XML] [Import into EndNote] [Citations on Google Scholar]

Research Paper

Use of Untreated and Autoclave-Treated Wheat Germ Meal in Growing Rabbit Diets.

Salama WA, Refaie AM, Amin HF and Abdel-Mawla LF.

World Vet. J. 9(3): 192-200, 2019; pii:S232245681900025-9
DOI: https://dx.doi.org/10.36380/scil.2019.wvj25

ABSTRACT

The present study was intended to investigate the influence of using 20% and 40% treated or untreated wheat germ meal in growing New Zealand rabbit diets. A total of 75 weaned New Zealand White rabbits aged six weeks old, with an average initial weight of 659.60±18.84g were divided into five groups with five replicates in each group (three rabbits per replicate). The first group was fed on a basal diet (T_1), second and third groups received diets containing Wheat Germ Meal (WGM), as replacement of soybean meal protein, at levels of 20% and 40% and were labeled as T_2, T_3, respectively. Fourth and fifth groups were fed with 20% and 40% autoclave-treated autoclaved WGM (T_4 and T_5, respectively). The trial was continued until 14 weeks of age. The present study was evaluated growth performance, blood parameters, carcass traits, meat quality in different groups and also economic efficiency was calculated. There were insignificant differences in terms of live weight, daily weight gain, carcass weight and dressing percentages among rabbits in groups of T_1, T_2, and T_4. Rabbits in the group of T_4achieved the best feed conversion ratio. Digestion coefficients of crude protein, crude fiber, ether extract, nitrogen-free extract, and nutritive value in terms of digestible crude protein, total digestible nutrition, and digestible energy did not significantly differ between T_1 and T_4. However, these factors significantly decreased in T3 and T_5 compared to T_1. Plasma total protein and globulin significantly increased in rabbits of T_2 and T_4 compared to those fed in T_1 group. A significant decrease in total cholesterol and total lipid for rabbits in groups of T_4, T_5, and T_2 was observed. Moreover, rabbits fed on T_4 or T_2diets had the highest economic efficiency. Conclusively, the untreated or autoclaved WGM can be used in growing rabbit diets up to 20% for replacing the soybean meal protein, which caused low feed costs without adverse effects on the growth performance of rabbits.

Key words: Rabbits, Soybean meal, Wheat germ meal

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Research Paper

Productive Characteristics and Reproductive Responses to Estrus Synchronization and Flushing in Abou-Delik Ewes Grazing in Arid Rangelands in Halaieb - Shalateen - Abouramad Triangle of Egypt.

Farrag B.

World Vet. J. 9(3): 201-210, 2019; pii:S232245681900026-9
DOI: https://dx.doi.org/10.36380/scil.2019.wvj26

ABSTRACT

There are a few reports about the reproductive aspects or uses of both of flushing and estrus synchronization in Abou-Delik ewes grazing in the South Eastern zone of Egypt. Thirty-three Abou-Delik ewes were allocated to three experimental groups (n = 11 in each) to study the effects of estrus synchronization and flushing on reproductive responses and productive characteristics under arid conditions of South Eastern zone of Egypt. Group one served as control represent the system dominant in the area (without estrus synchronization and flushing ration). Ewes in group two were estrous synchronized with two doses of $PGF_2\alpha$, 10 days apart without flushing ration. Ewes in group three were estrous synchronized just like the second group and received 300g of barley grain/head/day as flushing meal for three weeks before the start of breeding season. All ewes were grazed *Panicum turgidum* (natural vegetation dominant in the area) for eight hours daily. Results showed that, the percentage of estrus exhibition in group three reached 100%, while the lowest percentage was observed in group one (81.82 %). Estrus activity signs in synchronized groups, occurred in 70 and 81.81% during the first 48 h after the second dose of $PGF_2\alpha$, for groups two and three respectively, compared to control group (22.22 %). The onset of estrus was earlier in synchronized groups than control group. Duration of estrus did not differ significantly. Estrus intensity in group three was higher ($P< 0.05$) compared to the other groups. Conception and lambing rates were 100% in group three. Third group showed the highest insignificant litter size that was 18% higher than the other groups. The overall mean of birth weight, weaning weight and average daily gain of Abou-Delik lambs were 2.91, 16.89 and 0.116 kg, respectively. There is no significantly effect on concentrations of plasma progesterone among groups. While there were significant differences between sampling periods. In conclusion, under grazing on arid rangelands conditions in the South Eastern zone of Egypt, using flushing and/or estrus synchronization can be useful to improve reproductive and productive characteristics of Abou-Delik sheep.

Key words: Abou-Delik sheep, Estrus synchronization, Flushing, Productive performance, Rangelands, Reproduction

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Characterization of Pregnancy-Associated Glycoprotein as a Biomarker of Pregnancy in Etawa Crossbred Goat

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ABSTRACT

Pregnancy-Associated Glycoprotein (PAG) is secreted by the placenta, which is produced in mononucleate and binucleate trophoblast cells. The current research was conducted to find out a substance for diagnosing early pregnancy in Etawa crossbred goats. Six Etawa crossbred goats (not pregnant, three months pregnant and four months pregnant) were subjected in the present study from Livestock Government Institution Breeding in Singosari, Malang. The research methods consisted of sample collection, identification PAG with sodium dodecyl sulfate– polyacrylamide gel electrophoresis, the determination of concentration with Biuret method and specificity test with Western Blot assay. The obtained results showed that the molecular weight of PAG from pregnant Etawa crossbred goats was 55.85 kDa. The average concentrations of PAG in the goats of non-pregnant, three months pregnant, and four months pregnant were 1.83±2.98, 3.79±2.72 and 4.36±2.63, respectively. The results of the specificity test with the Western Blot molecular revealed a molecular mass of PAG was 55 kDa. The findings of the present study demonstrated PAG in Etawa crossbred goats can be used as an indicator of pregnancy.

Key words: Biomarkers, Etawa crossbred, PAG, Pregnancy

INTRODUCTION

Diagnosis of early pregnancy in goat can be done in two ways: through detection of specific substances in the peripheral circulation such as Pregnancy-Associated Glycoprotein (PAG) or non-specific substances in the blood, urine or milk such as progesterone and estrone sulfate (Hafez, 2000). PAGs are pregnancy indicators that are produced by mononucleate and binucleate trophoblastic cells (Perenyi et al., 2002; Karen et al., 2003; Sousa et al., 2006). Garbayo et al. (1998) purified three PAGs from goat placenta which differed in amino acid sequences and molecular weight (55 kDa, 59 kDa, and 62 kDa) and each of them had several isoforms with different isoelectric points. Isolation of ovine PAG was obtained at a molecular weight of 30.86 kDa from placental cotyledon (Setiatin et al., 2009). In cattle, PAG isolated from the blood serum during 274-279 days of gestation was characterized in molecular weight of 67.34 kDa (Lestari and Ismudiono, 2011). In livestock reproductive management, early pregnancy diagnosis is very economically advantageous in determining pregnancy status after mating (Restall et al., 1990; Goel and Agrawal, 1992). Generally, the length of the estrous cycle of the goat is around 21 days (Jainudeen et al., 2000). The economic losses of pregnant goats can be minimized or prevented by methods of early pregnancy diagnosis (Singh et al., 2004).

Pregnancy tests have the potential to be very suitable for field practice. PAG can be measured in maternal blood circulation (Shahin et al., 2013). Pregnancy is diagnosed using PAG test on day 24 of gestation (Reese et al., 2017). The knowledge of mechanisms involved in the production and control of PAG is beneficial in livestock breeding and facilitates diagnosis of pregnancy (Santos et al., 2018). Therefore, the current study was designed to evaluate blood serums for early pregnancy diagnosis in Etawa crossbred goats managed in intensive conditions in Indonesia.

MATERIALS AND METHODS

Ethical approval

This study was approved by the Institutional Animal Ethics Committee Brawijaya University (Code No.1108-KEP-UB).

Collection of Samples

The present study was conducted on six crossbred goat aged 3 to 4 years in Livestock Breeding Institution Government in Singosari, Malang, East Java, Indonesia. The analysis of blood samples was conducted at the Department

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of Veterinary Reproduction Airlangga University, Surabaya and Biosains Laboratory, Brawijaya University, Malang, East Java, Indonesia. The goats were maintained under intensive system of management in well-ventilated pens and dietary and management conditions were the same for animals. Blood samples were taken from jugular veins of non- pregnant and pregnant goats in the different gestational age (3-4 month). The serum samples were centrifuged at 3000 rpm for 10 minutes and the supernatant was transferred in a new tube and was stored at -20°C until further use.

Identification of PAGs with Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The running gel was inserted into the SDS-PAGE tool through the wall to below the top line. Then, 1 ml butanol was added and left for 25 minutes. After the gel freeze, butanol was removed and cleaned with PBS then dried with Whatman paper. The 12% stacking gel is inserted from the top of the wall until it was fully set for 25 minutes. The comb was inserted and the remaining gel was cleared with a buffer. The samples were mixed with 5 µl of liquid buffer and heated at 100°C for 5 minutes. Then samples were inserted into the mold hole with a tip of 200 µl. The mold is inserted into an electrophoresis gel device, the power supply at the start was 125 V, 40 mA for one hour. When the electrophoresis was completed, it was turned off and the plate was opened and separated, then the gel was stained with Coomassie Brilliant Blue (Merck, Germany). Molecular weight determined using standardized regression between relative migration and molecular weight markers. Then, it was tested for specificity with Western Blots test.

Examination of PAGs levels using the Biuret method

The not-colored SDS-PAGE gel was cut to the desired tape. Each gel was inserted into a nylon sack and packed in a glass block containing PBS while were mixed on a magnetic stirrer for 24 hours. PBS was replaced every 6 hours. The gel pieces were stained with silver staining to detect protein. The total protein concentration was determined using Biuret reagent by adding a standard solution of Bovine Serum Albumin (BSA) protein. The sample cuvette was prepared with a PAG and 2.5 ml of the Biuret reagent. The standard cuvette as filled with 0.05 ml BSA and 2.5 ml of the Biuret reagent. The blank cuvette was prepared by adding 2.5 ml Biuret reagent and 0.05 ml of distilled water. Three cuvettes were left for 30 minutes and color intensity was read by Bausch Lomb Spectronic Spectrophotometer at a wavelength of 540 nm.

Specificity test of PAG with Western Blot

Western blot was carried out by using fragments of PAG bands which had been run in SDS-PAGE and were transferred to the nitrocellulose membrane. The membrane was blocked with 3% BSA in 20 mM Tris-HCl at pH 7.5 and 150 mM NaCl for one hour then was incubated with the primary antibody (anti-PAG) diluted in Tris/NaCl containing 1% BSA. After washing with Tris-Cl containing 0.05% TWEEN 20, the membrane was incubated with secondary antibodies (anti-rabbit IgG labeled AP, 1:1000 dilution) and was added Western Blue Substrate (Promega, USA) (Aulanni'am, 2004).

Statistical analysis

The standard protein curve was made to obtain the molecular relative mass of the samples (Gaspersz, 1995). The relative molecular mass of each protein defined by data converted from relative migration distance (*Rf*) values according to following linear equation: Y = b0 + b1X; where Y is molecular weight (kDa), b0 is a constant, b1 is coefficient of relative migration, and X is relative migration of protein band.

The total protein concentration of PAGs by Biuret method was calculated as follows: $Y = 5.10^{-5}X$ Where Y is absorbance and X is a concentration of protein (µg/ml). The data of the Biuret method was statistically analyzed using ANOVA multivariate. Data were analyzed using SPSS version. 17.0 software (SPSS Inc, USA). A $p < 0.05$ were regarded as statistically significant.

RESULTS

The profile of the PAGs isolated from blood serum of Etawa crossbred goats using SDS-PAGE are shown in figure 1. The protein molecular weight was measured by relative migration when the protein passes through the separating gel (Figure 2). Then, based on the logarithmic equation (y = 2.401 + -1.4752X; R2 = 0.9829) obtained by calculating the relative migration, the molecular weight was obtained as a band that appeared on electrophoresis. The molecular weight of protein bands from six samples are presented in table 1. The serum of control and pregnant goats has the same protein profile. However, in blood serums of pregnant goats, there was a protein with a molecular weight of 55.85 kDa which was expected to be a specific PAG.

To ensure that the electroelution protein was a PAG, the elution results was examined by Biuret method to determine PAG protein levels. The results of the examination using the Biuret method can be seen as isolation of PAG in table 2. The protein concentration was the lowest in non-pregnant goats (1.83±2.98), then 3 months pregnant (3.79±2.72)

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and showed the highest value in 4 months pregnant (4.36±2.63). The indicated correlation in blood serum had a significant difference (P<0.05).

Specificity tests were carried out to ensure that the detected protein was PAG. The results of the Western Blot test showed purplish bands on nitrocellulose membranes with a molecular weight of 55 kDa (Figure 3). This finding proved that the visible bands were PAG molecules. The molecular weight can be read using a reference marker protein with a molecular weight range of 15 to 260 kDa. Protein band with a molecular weight of 55 kDa was found in samples of pregnant goats in gestational age 3 and 4 months, whereas in samples of non-pregnant goats there was a protein band with a molecular weight of 23 kDa.

Table 1. Molecular weight of protein obtained from blood serum of Etawa crossbred goats

Serum Sample	Protein Molecular Weight (kDa)											
	15.29	**20.23**	**24.10**	**31.89**	**39.35**	**48.55**	**55.85**	**62.04**	**82.10**	**120.68**	**165.39**	**197.03**
Non-pregnant	+	+	+	+	+	+	-	+	+	+	+	+
3 month pregnant	+	+	+	+	+	+	+	+	+	+	+	+
4 month pregnant	+	+	+	+	+	+	+	+	+	+	+	+

(+) = positive band; (-) = Negative band

Table 2. The average concentration of PAGs isolated from blood serum of pregnant Etawa crossbred goats by using Biuret method

No.	Sample	Absorbance 1	Absorbance 2	Average concentration (µg/ml)
1	non-pregnant	1.48	2.18	[a] 1.83±2.98
2	3 month	5.22	2.36	[b] 3.79±2.72
3	4 month	2.45	6.23	[c] 4.36±2.63

Different superscript letters indicate significant differences (p<0.05); PAG: Pregnancy-Associated Glycoproteins; Absorbance was read in a spectrophotometer at a wavelength of 540 nm

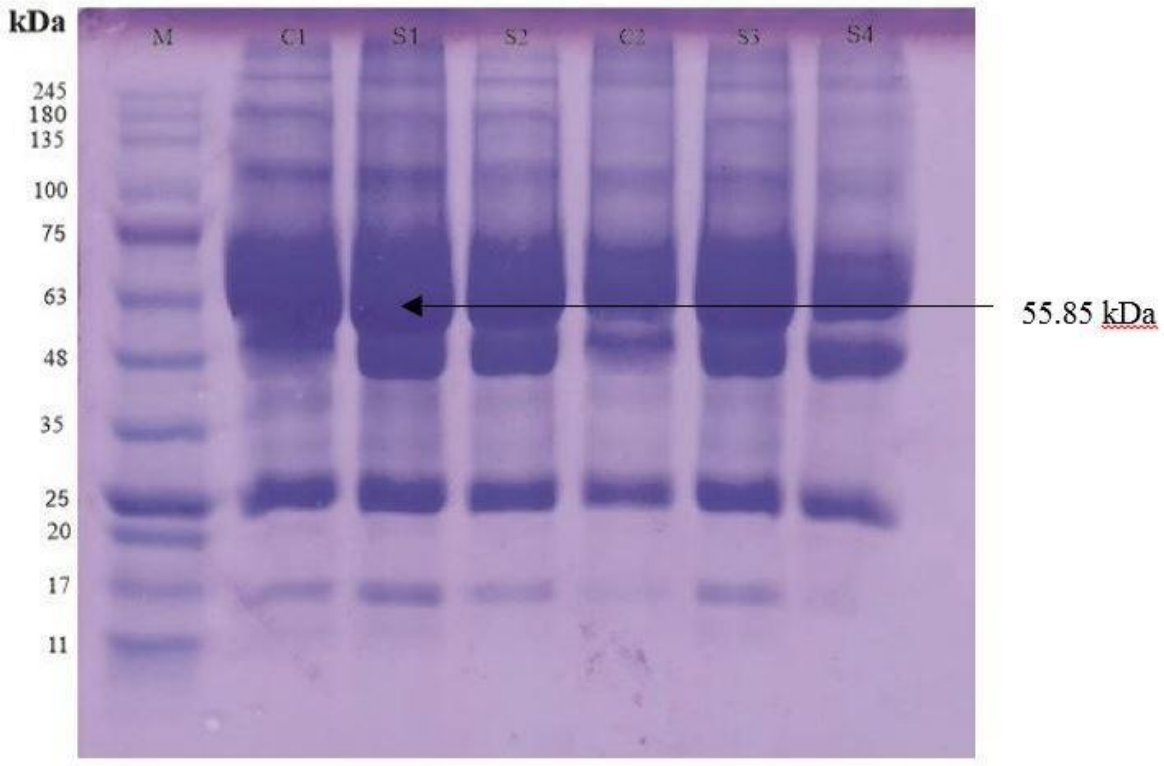

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Figure 1. This is the SDS-PAGE analysis of PAGs isolated from blood serum of Etawa crossbred goats. Lane M: Tris-Glycine 4-20%, 11-245 kDa as marker; Lane C1 and C2: non-pregnant; Lane S1 and S3: 3 months pregnant; Lane S2 and S4: 4 months pregnant.

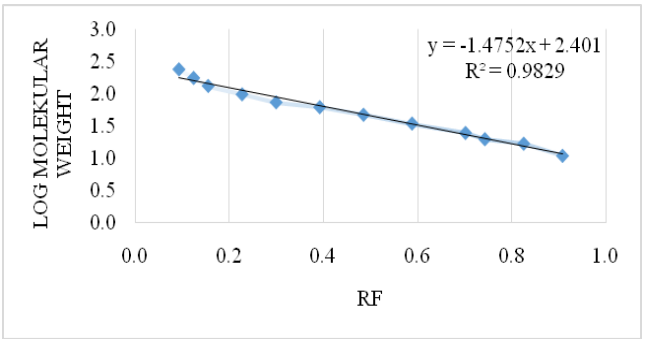

Figure 2. Determination of molecular weight by calculating the relative migration

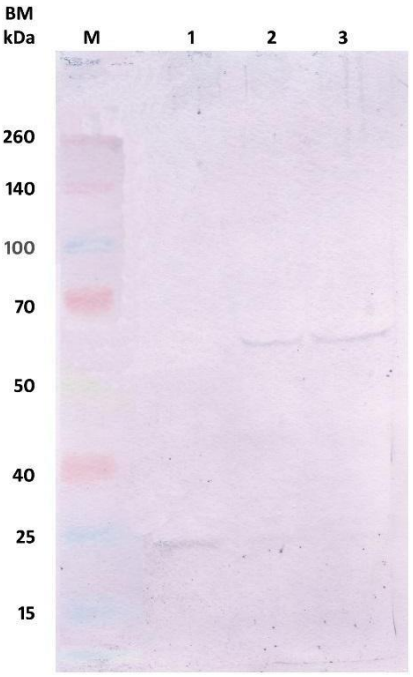

Figure 3. The Western Blot analysis for PAG isolated from blood serum of Etawa crossbred goats. Lane M: marker; Lane 1: non-pregnant; Lane 2: 3 months pregnant; Lane 3: 4 months pregnant.

DISCUSSION

According to the obtained results in the present investigation, the molecular weight of PAG in Etawa crossbred goats was 55.85 kDa. This finding is in accordance with the discovery of caprine PAG in the previous study by Amiri et al., (2004) that identification protein was performed by SDS-PAGE and found the molecular weight of caprine PAG was between 55 to 66 kDa. Moreover, Garbayo et al. (1998) reported different molecular mass (55 kDa, 59 kDa, and 62 kDa) for caprine PAG. PAGs can be detected in the maternal blood circulation from embryo implantation (Gordon, 1999). Trophoblast placental cells are present in blood circulation during implantation until parturition and are responsible for producing PAGs throughout the gestation period (Gonzales et al., 2000).

The protein concentration of PAG increases progressively at 3 and 4 months of gestation. Blood PAG levels steadily increased during early pregnancy in goats (Singh et al., 2019). Ispierto et al., (2016) reported PAG concentrations were significantly higher in twins compared to single pregnancies. The current research is in agreement with one of the statements by Cavanagh (1996) who mentioned PAG was first discovered as a substance-related to pregnancy and was detectable in 6-24 hours after conception in all species such as rats, humans, pigs, and sheep. Duplants (2000) declared that PAG was detected after the implantation and remained in the pregnant goat until parturition and disappeared after the birth process. Many factors influence the concentration of PAG, such as breeding (Ranilla et al., 1994; Guilbault et al., 1991), the number of fetuses (Benitez-Ortiz, 1992) and *in vitro* culture period (Ectors et al., 1996). Therefore, differences in PAG expression observed in the present study can be related to variations in breeds, procedures, and geographical location.

The results of present study showed the protein bands on nitrocellulose membranes, indicating a specific bond between PAG antibodies and PAG antigens isolated from Etawa crossbred goats pregnant. The further production of PAG increases the bond between PAG antibodies and PAG antigens and provides thicker protein bands. This finding is supported by Aulanni'am (2004), in Western Blot method, PAG antibodies recognize PAG antigens as specific antigens and bind together thus purplish-colored protein bands become visible.

CONCLUSION

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The present study characterized PAG with a molecular weight of 55.85 kDa in Etawa crossbred goats at 3 and 4 months before parturition. Moreover, application of PAG as a biomarker of pregnancy was confirmed in Etawa crossbred goats.

DECLARATIONS

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Consent to publish

All authors contributed to write and publish manuscripts in the World's Veterinary Journal.

Competing interests

The authors declare that they have no competing interests.

Author's contribution

IKN wrote the manuscript and conducted the research, TDL conceptualized the research, and HAP revised the final form of the manuscript.

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